TRYBLIOGRAPHA DACI WELD (HYMENOPTERA: CYNIPIDAE): BIOLOGY AND ASPECTS OF THE RELATIONSHIP WITH ITS HOST ANASTREPHA SUSPENSA (LOEW) (DIPTERA: TEPHRITIDAE)

By

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Trybliographa daci, a cynipid parasitoid, was introduced to Florida as a possible biological control agent for the Caribbean fruit fly Anastrepha suspensa (Loew). This is a report of its biology and relationship with its host in the laboratory. Development of T. daci in 2nd instar host larvae required 27 - 33 days at 27.5°C, 50-60% RH and 14 hr light. Significantly greater numbers of parasitoids survived to the adult stage when 70% RH and 50% moisture (W/W) in the pupation medium were maintained. Characteristics and duration of eggs, 4 larval instars and pupae were described.

The host often encapsulated the parasitoid, with melanization of host hemocytes occurring around the chorion of the parasitoid egg within 72 hr after parasitization. First instar parasitoid often successfully evaded host defenses and continued development. Incidence of encapsulation was higher in hosts that were 4-, 5- and 6-days-old, and in

superparasitized hosts. The life cycle of hosts that successfully encapsulated the parasitoid was not affected and flies containing 2 or 3 capsules successfully emerged.

Adult parasitoids lived 23 days at 24°C and 60% RH. Higher temperature and lower RH caused premature mortality, but they lived up to 56 days at 20°C and 60% RH. Female parasitoids had high fecundity. They mated only once and reproduced by arrhenotoky. Five-day-old females oviposited in 1 through 6-day-old host larvae and discriminated between parasitized and unparasitized hosts. Younger hosts (1st and 2nd instar) gave rise to larger numbers of parasitoids than older hosts (3rd instars), but parasitoids emerging from 3rd instar hosts were larger. Increased parasitoid numbers with a constant number of hosts increased superparasitism and host and immature parasitoid mortality. Each female parasitoid laid fewer eggs due to interference from other females, and also restrained oviposition when suitable hosts were not available. Oviposition by female parasitoids increased with increasing 2nd instar host numbers. Individual 5-day-old females laid a mean of 72 eggs per day when 100 hosts were available, but only 49 eggs per day when provided with 25 hosts. Parasitoid progeny (= $\frac{\text{Progeny recovered/female/day}}{\text{Eggs laid/female/day}} \times 100$

was equal to 24.7%, 78.4%, 88.4% and 89.1% at host densities of 25, 50, 75 and 100, respecively. Second instar host larvae and host:parasitoid ratio of 75:1 are recommended for mass rearing of T. daci.

INTRODUCTION

The Caribbean fruit fly, Anastrepha suspensa (Loew) (Diptera: Tephritidae), is indigenous to the West Indies, and was first introduced into Florida in 1931. The fly exhibits great adaptability, and now is known to infest more than 80 species of host plants (Greene 1934, Weems 1965, Swanson and Baranowski 1972, Chambers 1977). The damage is done by the larvae as they tunnel in the fruit and thus render it unfit for human consumption. The fly represents a threat to the citrus industry in Florida, although citrus is not a favored host.

Chemical control of the Caribbean fruit fly in Florida is not practical for several reasons. The fly has spread widely over the southern and central part of the state; it is a backyard and urban pest; and an exceptionally good attractant is not available. An important aspect of research in control of the fly is biological control through importation of potential parasitoids and predators, and their subsequent rearing and release.

Trybliographa daci Weld is a larval-pupal parasitoid of several species of the genus Dacus (Diptera: Tephritidae) in southeast Asia and Australia (Weld 1951, Clancy et al. 1952). The parasitoid was recently imported by Dr. R.M. Baranowski to the AREC (Agricultural Research and Education Center), Homestead, Florida, from IRAT (Institute de Researches Agronomiques Tropicales et des Culturales Vivrieres), Antibes, France, and was reared for several generations in A. suspensa larvae but was not

recovered from wild A. suspensa after several field releases. It was also introduced to Hawaii and successfully reared in Ceratitis capitata (Wiedemann) and Dacus dorsalis (Hendel) (Clausen et al.1965), but did not become established in the field. Recently it has been reared in C. capitata and released against that pest in France. At the present time there is insufficient data to ascertain either the complete life cycle or the biological control potential of T. daci.

Some of the most important aspects of biological control and mass rearing of imported parasitoids relate to specific host:parasitoid relationships. Difficulties in establishing and rearing parasitoids may arise in the immune reactions of new hosts or from the failure of the female parasitoid to discriminate between parasitized and unparasitized hosts. Superparasitism and consequent waste of eggs by the female parasitoid may result from inadequate rearing procedures. Research into these potential problems is an important component of bio-control programs. In cases where the parasitoid failed to control the pest (host) there is a remarkable lack of studies on the subject of host:parasitoid relationships. This may be explained partly by the fact that pest control specialists need to obtain immediate results. In such apparent failures, the rejected parasitoid species was only studied briefly and its potential to control the intended, or other potential hosts was not fully explored. Detailed studies to determine why the parasitoid failed to become established may generate information useful in future bio-control work.

The following research was undertaken in order to study the basic biology of T. daci in A. suspensa larvae and to explore aspects of the

host:parasitoid relationship useful in improving mass rearing methods.

The objectives were to

1. Describe the developmental stages of T. daci and the changes in the host and the parasitoid during the parasitization process.

2. Describe courtship, mating and oviposition behavior and mode of reproduction of the parasitoid.

3. Determine the level of relative humidity necessary to optimally maintain the parasitoid colony.

4. Evaluate the effect of host age upon the development and characteristics of the parasitoid and to select the optimal host age for parasitization.

5. Evaluate the effect of parasitoid and host densities on parasitoids egg distribution and developmental success of parasitoids as a function of host availability.

CHAPTER I

GENERAL REVIEW OF LITERATURE

The Host:Parasitoid Relationship; General Aspects

The female parasitoid may emerge in a habitat from which hosts have moved or disappeared, and she then must seek a suitable environment for her progeny. Based upon the observations of Salt (1935), Doutt (1964) divided the process that resulted in successful parasitism into four steps: (a) host habitat location, (b) host location, (c) host acceptance, (d) host suitability. Vinson (1975) added an additional category, that is, (e) host regulation. The first 3 categories are considered to be integral components of the host selection process (Vinson 1976). The last 2 steps (host suitability and host regulation) describe those factors that result in successful development of the parasite in the host.

Host Selection Strategy (Host Habitat Location, Host Location, and Host Acceptance)

It has been suggested that hosts are found by parasitoids through random searching once a suitable habitat has been located (Vinson 1976). However, there is evidence that searching is not completely random but modified through host related cues recognized by the parasitoid. Whether these cues are perceived independently or in a hierarchial order, each succeeding step reduces the distance between it and its host and

increases the probability of encounter with the potential host (Vinson 1976, 1977a).

Before the encounter, the female parasitoid looks for a particular environment irrespective of the presence of hosts (= host-habitat finding). Generally this preferred habitat is also the habitat for her specific host (Doutt et al. 1976). The selection of that area is influenced by physical factors, as well as by chemical substances emanating from the host and/or the host food. These cues vary with the insect species and act as long-range cues. The nature of the signals, their origin and mode of action have been studied in several species (Vinson 1976). The location of the host (= host finding) is mediated through short range cues emitted by the host or associated with its activity (Vinson 1975, 1976, Greany et al. 1977c). The chemical stimuli involved in host selection have been defined on the basis of their origin and the behavioral response that they elicit (Brown et al. 1970). Among the cues kairomones are of primary importance. Kairomones have been defined as chemicals produced or acquired by an individual of one species, which when contacted by an individual of another species evoke in the receiver a behavioral or physiological response adaptatively favorable to the receiver (Nordlund and Lewis, 1976). Kairomones and other chemical or physical short range cues stimulate searching activities but are not responsible for the acceptance of the host and egg deposition (Vinson 1976, Doutt et al. 1976).

After the host has been located, the parasitoid may accept it as a suitable site for parasitization (= host acceptance). The acceptance of a host has been attributed to a number of factors that are difficult to

separate from those leading to host habitat and host finding. Chemicals are also important as are shape, size, texture, sounds and electromagnetic radiation. These factors influence the reflex action of piercing or probing which could culminate in egg release, if the host is present (Vinson 1975, 1976).

Many examples can illustrate the complexity of factors in the host selection process; each factor may act independently or influence other steps. The degree of influence is often difficult to discern. Ichneumonid parasitoids of aphidophagous syrphidae, for example, are attracted to their syrphid host by aphids. Pre-oviposition behavior is caused by contact with chemicals present in the larval cuticle. Movement was the final cue for ovipositor insertion of Enizeum ornatus (Grav) (Ichneumonidae), which oviposits in the cephalic ganglion of the host Metasyrphus luniger Meigen (Syrphidiae) (Rotheray 1981). The final cue for egg release was not found.

Biosteres longicaudatus (Ashmead) (Braconidae) parasitizes only mobile A. suspensa larvae. This fact and the positive response of the female parasitoid to artificially produced vibrations implicate the combination of movement and vibrations in the location of the host (Lawrence 1981a).

The size and age of hosts are usually related, but each factor may influence the host choice independently. The changes in acceptability influenced by age have been related to physiological alteration of the internal or external factors necessary for acceptance (Bakker 1971, Vinson 1975). The degree of importance of these and other factors is variable and specific, and their study requires behavioral, ecological and physiological analyses.

The above mentioned factors are parameters in one aspect of host acceptance, that is, the identification of the host. A second step is host discrimination, which occurs when the female parasitoid distinguishes between parasitized and healthy hosts. Salt (1934) first described this property in Trichogramma evanescens Westood. Salt (1937) demonstrated the presence of marking substances left on the surface of the parasitized host's eggs that deterred further oviposition. Apparently these substances were not permanent and they could be washed from the egg surface. After they were no longer effective, a second female parasitoid could still distinguish the parasitized host after the insertion of the ovipositor. The ability to detect an already parasitized host after inserting the ovipositor is exhibited by many species of parasitic Hymenoptera and has been associated with internal marking (Salt 1961, Vinson 1976).

The frequency of marking substances is high, although the origin and nature have been investigated in only a few cases (Vinson 1976). Whatever their composition or origin, they are important in the prevention of superparasitism and/or multiple parasitism (Doutt et al. 1976), but they also act as kairomones for cleptoparasitoids (Vinson 1976). Parasitic Hymenoptera are commonly solitary species, that is, they require one host for the development of one individual. Superparasitism results when more than one egg of the same species is laid in the same host (Askew 1971). Multiple parasitism is defined by Doutt (1964) as the "simultaneous parasitization of a single individual host by 2 or more different species of primary parasites" (p. 124).

The rejection of previously parasitized hosts after ovipositor insertion has been associated with changes in the host hemolymph perceived

through chemoreceptors (Fisher and Ganesalingam, 1970). The existence of sense organs on the ovipositor was first postulated by Fulton in 1933 (cited by Fisher, 1971) and the structure of those sensilla has been studied in several species; e.g. Greany et al. (1977b) described 2 types of sensilla on the ovipositor of B. longicaudatus. Host discrimination in Pseudeucoila bochei Weld (Cynipidae) is not accomplished through external markers, but possibly through the ovipositor's sensilla located in the base of the valves (Lenteren 1972). Other cues used as criteria for rejection of the host may include movement, heart beat, sounds or vibrations, but most of these remain unproven (Fisher 1971).

Host Suitability

Upon acceptance and actual oviposition in or on the host, the host must meet the requirements for successful development of the parasite progeny, that is, it has to be suitable. Salt (1938) defined a suitable host as one in which the parasite can generally reproduce fertile offspring. Suitability refers to the nutritional and physiological state of the host; indeed, it is related to the environmental factors which, in total, directly affect the parasite life cycle or influence host defenses (Vinson 1977b). Many factors may influence nutritional suitability of the host, a concept which includes not only the level and quantity of nutrients but growth factors (Vinson 1980). These factors are generally related to host age, size, nutritional history, and genetic composition. The successful development of the parasitoid progeny to adult stage in the host depends on factors related to the parasitoid itself, including evasion of or defense against the host immune system, and competition

with other parasitoids present within a single host.

Host defenses. Insects can actively interfere with the development of a parasitoid by the reaction of the immune system (Vinson 1980, Nappi 1975a). There has been little work done with regard to possible humoral response to parasitoids, but extensive studies support the function of the blood cells or hemocytes in the elimination of foreign bodies by phagocytosis and/or encapsulation (Vinson 1977b, Salt 1963). Salt (1963) pointed out that insects usually respond to metazoan parasites through encapsulation "whereby the hemocytes become attached to the foreign body surface, flatten out and form layers which often become melanotic" (p. 557).

The process of capsule-formation in insects has been described by several workers (Salt 1970); however, the physiological control that culminates in the formation and melanization of the capsule is not known (Nappi 1975a, Radcliffe and Rowley 1979, Salt 1968).

There are 2 major classes of capsules: cellular and humoral. Only cellular are produced against parasitoids and in general they are similar in basic structure (Radcliffe and Rowley 1979). In many species the inner layer of the capsule eventually undergoes melanization. In some insects melanization of the capsule is precocious. In the latter case a relatively thin layer of cells covers the parasite and quickly becomes melanized. These capsules are called sheath type (Salt 1963, 1970).

A sheath type of capsule was described by Walker (1959) in Drosophila malanogaster (Meigen) parasitized by P. bochei and had been found in another cynipid, P. mellipes Say by Streams and Greenberg (1969). The capsule around P. bochei is formed by modified plasmatocytes, called lamellocytes. These are flat blood cells that normally appear in

non-parasitized Drosophila larvae at the time of pupation, but they develop precociously in parasitized host larvae (Rizki 1957).

Several hypotheses have been proposed to explain the activation of the cell-mediated responses in insects. Nappi (1975a) analyzed them and advanced 2 hypotheses. The first hypothesis considers the initiation of the encapsulation process by fortuitous contacts with the non-self materials. The fact that any inert object can elicit encapsulation does not prove that recognition results only by contact stimuli. In some cases "injury factors" from damaged tissues may increase the mobility or adhesiveness of the blood cells and cause them to aggregate at the site of injury or around the foreign body that probably becomes contaminated with the injury factors. The second hypothesis is based on changes of the normal titer of host hormones induced by parasitization. This may change cell permeability to certain metabolites (such as tyrosine and/or tyrosinase) and causes premature differentiation and migration of hemocytes. Histological changes in blood cells due to parasitism have been studied in a variety of host:parasite systems (Nappi and Stofolano 1971, Nappi and Streams 1969, Takei and Tamashiro 1980).

Studies suggest parasitoid modifications or influences upon host endocrines. Neck-ligation of D. algonquin Steterbant to exclude the neuroendocrine glands from the hemocytes and the parasitoid P. bochei resulted in the reduction of encapsulation (Nappi 1973). Opposite results were observed by Rizki (cited by Riddiford 1975), who induced precocious transformation of hemocytes into plasmatocytes as a consequence of ligation. Riddiford (1975) pointed out the possibility that the host response to

the parasite entry may trigger the appropriate changes in the host hemocytes. These changes could arise through the direct action of the "injury factors" in the blood cells or indirectly through particular changes of the prothoracic glands. For those changes to occur the hormonal milieu of the host must be proper, especially in levels of Juvenile Hormone (JH). High levels of JH in 1st and 2nd instar larvae of the host could prevent hemocyte transformation.

Evasion of host defenses. The mechanisms used by parasitoids to evade host defenses differ according to the specific host:parasitoid system. Some parasitoids may evade host defenses by acquisition of host materials which form a coat around the parasitoid that results in the failure of the host to recognize the parasitoid as foreign (Salt 1968, 1970, Vinson 1977b). The chorion structure plays a very important role in the recognition of non-self materials by the host blood cells (Rotheram 1967, Vinson and Scott 1974). A coating on the parasitoid egg may prevent encapsulation.

Salt (1973) provided evidence that eggs of Nemerites (=Venturia) canescens (Grav) were resistant to the cellular defense reaction of Ephestia kuehniella Zeller due to a coating acquired in the lateral oviducts of the female parasitoid as demonstrated later by Rotheram (1973).

Kitano (1969) in his extensive work tested the possible factors involved in the inhibition of encapsulation of Apanteles glomeratus L. by Pieris rapae L. He (Kitano 1969) supposed that the parasitoid eggs passing through the lateral oviducts were provided with a secretion that inhibited encapsulation. The first and second larval instars were protected by the "giant cells" or teratocytes. These also prevented the

reaction of the host hemocytes against the disposed chorion. The physical properties of the serosal membrane were considered important, but whether the inhibition of encapsulation was due to specific secretions or to physicochemicals properties was not proven.

Recent studies have demonstrated the presence of virus in the lateral oviducts of several ichneumonid parasitoids, e.g. a virus was found in Campoletis sonorensis (Cameron) that suppressed the encapsulation of the parasitoid egg by the host Heliothis virescens (F) (Edson et al. 1980).

Parasitoids can actively prevent encapsulation by injection of toxins that interfere with or suppress the hemocytic transformation (Salt 1968, Vinson 1977b). Pemberton and Willard (1918), for example, postulated that the inoculation of a substance by Opius fletcheri Sil. in D. cucurbitae Coquillett prevented the encapsulation of Tetrasticus giffardianus Sil. Streams and Greenberg (1969) suggested the injection of a substance by the female parasitoid P. bochei in its natural host, D. melanogaster, that prevented encapsulation. The same substance can protect P. mellipes, a species normally encapsulated by the same host species. For that protection to occur both parasitoids must oviposit within a critical time interval.

Occasionally partially encapsulated parasitoids may get free from the adherent hemocytes by physical movements, but whether the parasitoid can survive is uncertain (Salt 1968).

Host Regulation

The successful development of a parasitoid depends largely on the ability or inability of the parasitoid to regulate the host's physiology

(Vinson and Iwantsch 1980). Parasitoids exhibit many different manifestations that interfere with the host's normal development and which benefit the parasitoid. These effects are referred to as host regulation (Vinson and Iwantsch 1980). Parasitized hosts may exhibit morphological, physiological or behavioral changes, caused at the moment of parasitization or during parasitoid development. Reduction of total weight was proven in Manduca sexta (L.) after parasitization by A. congregatus L. In addition to reduction of growth and food consumption, parasitized larvae have a supernumerary sixth-instar, which suggests prevention of normal metamorphosis (Beckage and Riddiford 1978). Electrophoretic studies of changes in the hemolymph of H. virescens (F.) parasitized by Cardiochiles nigriceps (Viereck) demonstrated hydrolysis of proteins to free amino acids and formation of different proteins necessary for the normal growth of the parasitoid (Vinson and Barras 1970).

It is difficult to separate aspects of host suitability from host regulation. This is particularly true when nutritional suitability and evasion of host defenses are considered. These last factors are included in host suitability (Vinson 1980) but may result from changes in the hemolymph induced by the ovipositing female. Ultimately these factors favor the development of the parasitoid progeny.

The Caribbean Fruit Fly Anastrepha suspensa (Loew) (Diptera: Tephritidae)

The Caribbean fruit fly is a member of the family Tephritidae (= Trypetidae). This family is distributed in tropical and subtropical areas (Christenson and Foote 1960). The genus Anastrepha Schiner is indigenous to the southern United States, Mexico, Central and parts of

South America and the Caribbean Islands. Stone (1942) described the range of the genus as between 27° N and 35° S; it attains the greatest number of species within the tropics. Distribution of the few species detected in the United States of America is restricted to Texas, California and south Florida.

The taxonomic status of the genus has been reviewed several times and several keys are available. Greene (1934) included 54 species and Stone (1942) recognized 126. A new key had been published by Steyskal (1977), who recognized 155 species. Some keys are restricted to local fauna, e.i., Korytkoski y Ojeda (1968) published one for species in the Northeast of Peru. The taxonomy of the group has been difficult, especially because of the dynamic processes of speciation that give rise to new races with characters difficult to recognize by conventional taxonomy. Recently attempts have been made to use electrophoretic analysis to classify the phylogenetic relationships of Anastrepha species in Brasil (Morgante et al. 1980).

A. suspensa was first described by Loew in 1862 and given the name Trypeta suspensa from specimens collected in Cuba (Greene 1934). Included below are the names and synonyms that have been used to describe A. suspensa:

Trypeta suspensa Loew, 1862

(Trypeta) Acrotoxa suspensa (Loew), 1873

Anastrepha unipuncta Sein, 1933

Anastrepha longimaculata Greene, 1934

Origin, Distribution and Economic Importance

A. suspensa is found in Jamaica, Haiti, Dominican Republic, Puerto Rico, Cuba and Southern Florida (Weems 1965, Greene 1934). The species was first reported as an introduction in Florida in 1931, but it did not seem to become established. Detectable populations again were found in 1959 with the intensive trapping of the Medfly, C. capitata. From an infestation detected in 1965 at Miami Springs, the species spread rapidly over southern and central Florida and occasionally has been trapped close to Jacksonville (Chambers 1977, Weems 1965, Swanson and Baranowski 1972). More than 80 host plants from 23 families have been reported but most are not considered economically important. It has been reported from 11 species of the genus Citrus, and there is the threat of invasion of commercial citrus, because the species has demonstrated great adaptability to new situations. The threat to citrus has had an impact upon the exportation of citrus fruits to national and international markets. About 25% of the Florida grapefruit crop is annually sold in Japan, and the Japanese importers require fumigation of the cargo (Chambers 1977).

Both basic and applied research has been directed at the fly, with the research being supported by the citrus industry, USDA, the University of Florida and the State of Florida.

Basic Biology

The biology of the fly has been studied from laboratory-reared and wild populations. Mass-rearing techniques were studied by Burditt et al. (1974). Several artifical diets were tested using different base media,

with the objective of finding one that could be prepared and stored economically. The sugar cane bagasse diet developed by R.M. Baranowski (unpublished) has been used with good results for several years. The optimum temperature for mass rearing and the effect of physical conditions on the immature stages were studied by Prescot and Baranowski (1971). Lawrence (1979) extended these studies and described the morphology and duration of the immature stages at 27.5°C. The male and female reproductive systems were described by Dodson (1978). Studies on egg development and ovary growth under laboratory conditions have been concluded (Nation, personal communication).

One of the most important contributions in the last 10 years has been description of the sex-pheromone and pheromone-related behavior. Males from both mass-reared and wild strains of Caribbean fruit fly produce a sex pheromone that attracts mature females. In the initial studies of mating Nation (1972) developed a laboratory bioassay for the sex pheromone. A field bioassay was developed by Perdomo et al. (1975, 1976). Nation (1975) isolated the sex pheromone blend and partially elucidated its chemical composition (Nation 1982). Studies of mating interaction between laboratory-reared and wild flies were conducted by Mazomenos et al. (1977).

Control Measures

Chemical control was attempted through the application of organophosphate insecticides with or without baits. A trial erradication program using the sterile-male technique was undertaken, and release of

sterile insects in Key West demonstrated a good possibility for the use of this approach as a means of control (Burditt et al. 1974). Parallel with this program, a system for monitoring natural and sterile populations was initiated, and trap technology has been improved with the introduction of different types of traps, colors and food attractants (Greany et al. 1977a, 1978). In addition to efforts at chemical control and trapping, there was immediate interest in developing methods of biological control.

Biological Control

The exploration of the use of biological control agents was initiated in 1967. After systematic collecting in South Florida several species of Hymenoptera parasitoids were found. The most important were Spalangia cameroni Perk, S. endius Walker, (Spalangidae), Pachycrepoideus vindemiae (Rond) (Pteromalidae) and Parachasma anastrephillus Marsh (Brachonidae). The Hemipterous predator Fulvius imbecilis Say (Miridae) and Xylocoris galactinus (Fieb.) (Anthocoridae) were also present. As expected with an imported pest, the level of natural control by populations of these parasitoids and predators was very low (Baranowski and Swanson 1971).

Several parasitoids of fruit flies have been introduced into Florida. From the initial importations in 1969 Aceratoneuromyia indicum (Silv), B. longicaudatus (Ashmead) and Parashasma cereus (Gaham) were successfully reared under laboratory conditions. These species were released in the field, but follow up recoveries demonstrated that P. cereus was the most promising species (Baranowski and Swanson 1970). This species is now established in the field but present in low numbers (Baranowski

1980, personal communication). In the last few years B. longicaudatus has been recovered from the field more frequently than other parasitoids, and numerous studies of the biology, behavior, morphology and mass rearing methods for this parasite have been conducted (Greany et al. 1976, Lawrence et al. 1976, 1978, Lawrence 1981a,b). Another potential parasitoid T. daci was imported from IRAT (Institut de Researches Agromomiques Tropicales et des Cultures Vivrieres), Antibes, France, and has been reared in the laboratory successfully (Baranowski, unpublished). Unfortunately none have been recovered from the field following several releases.

There is agreement among plant protection workers for the necessity of a continuous search for pest management programs on the basis of ecological principles. It is thus expected that research in biological control will continue for several years.

The Parasitoid: Trybliographa daci Weld (Hymenoptera: Cynipidae)

Taxonomic Status

In contrast with the literature available on Anastrepha suspensa, very little information has been published in relation to T. daci which belongs to the superfamily Cynipoidea. This taxon includes phytophagous and entomophagous species. Members of the former group are gall makers and are placed in the family Cynipidae, subfamily Cynipinae. The entomophagous cynipids belong to the subfamilies Ibaliinae, Figitinae, Eucolinae and Charipinae (Clausen 1940). However, some authorities refer to these groups as separate families (Havilan 1921, James 1928, Askew 1971, Rotheray 1979). The species of Ibaliinae are parasitoids

of wood wasps (Askew 1971, Clausen 1940). The Figitinae and Eucolinae are recognized as parasitoids of young dipterous larvae (James 1928), and the Charipinae are superparasites of aphids through their braconid parasitoids (Askew 1971). Weld cited by Rotheray (1979), recognized another subfamily, the Aspicerinae, included earlier in the family Figitidae. Representatives of this subfamily are specific parasitoids of Syrphidae larvae. The subfamily Eucolinae includes the genus Tryblio-grapha and was reviewed by Ashmead (1903), who recognized 97 genera based on South American specimens. Ashmead (1903) referred to the genus Cothonaspis Hartig as a synonym of Trybliographa Forster.

Origin and Host Range

The species T. daci was described by Weld (1951) from specimens reared in Dacus umbrosa F. (Tephritidae) collected in Malaysia. It also has been reared in D. jarvisi (Tryon), D. dorsalis (Hendel) and D. tryoni (Froggatt) from specimens collected in Australia (Weld 1951, Clancy et al. 1952). The percentage of parasitization of D. dorsalis samples collected from Borneo and Malaysia was very low (Clausen et al. 1965, Clancy, 1950). T. daci was imported into Hawaii as a possible control agent for the imported pest, D. dorsalis. It was easily reared in D. dorsalis and C. capitata, the Mediterranean fruit fly, but failed to develop in D. cucurbitae (Marucci and Clancy 1950, Clancy 1952). After several releases in the field during 1949 to 1951, it was not recaptured (Weber 1951, Bess et al. 1961, Clancy et al. 1952).

The strain reared in Homestead, Florida, was imported from Antibes, France, where it had been reared successfully in Med fly larvae, and

released in the field against the same pest. Unfortunately there is no published information on rearing methods or on the biological and physiological aspects of the host:parasitoid relationship (Pralavorio 1980, personal communication).

Biology and Behavior

T. daci is a protelean larva-pupal parasitoid. The free living adults lay their eggs in larvae of their host and the progeny emerge from the host puparia (Weld 1951). The morphological characteristics of the eggs and of first instar and mature larvae were described by Clausen et al. (1965), whose primary objective was to recognize forms collected in the field. The same authors observed searching behavior by adults in the field. There is no published information on duration and description of the complete life cycle.

Importance of Cynipid Parasitoids

Askew (1971) has stated that "parasitic cynipids, with few exceptions, have been neglected by entomologists" (p. 160). Perhaps among the better studied cynipids have been species of the genus Pseudeucoila. These are parasitoids of Drosophila larvae. One of the more extensively studied is P. bochei. This species has been included in the program of ethological and ecological studies of parasites at the University of Leiden, the Netherlands (Lenteren 1976a, 1976b, Lenteren and Bakker 1978, Lenteren and Alphen 1978). The dynamic coevolutionary relationships between P. bochei and its hosts' defense reaction have been investigated since 1953 (see Reviews by Nappi 1975a and Salt 1963, 1970). The studies of P.

bochei and P. mellipes in this field have generated a hypothesis related to the active avoidance of host defenses by the female parasitoid (Streams and Greenberg 1969, Nappi 1975a). The changes in the host hemolymph after parasitization have been studied by Nappi (1975b) and Nappi and Streams (1969). The life history and reproductive capacity was studied by (Chabora et al. 1979, Kopelman and Chabora 1978). Studies on other species have been restricted to basic biology and morphology (Keilin et de la Baume-Pluvinel 1913, Havilan 1921, James 1928, Simmonds 1952).

CHAPTER II

DEVELOPMENTAL BIOLOGY OF TRYBLIOGRAPHA DACI

The importance of the study of insect development has been emphasized in many scientific papers. Morphological description is a useful tool in the evaluation of biological control programs, since many samples collected in the field contain immature forms that must be correctly identified and that are often difficult to rear under laboratory conditions (Clausen 1940, Hagen 1964, Rosen and DeBach 1973). The number of instars, morphology, duration of stages, and the description of the basic relationships between a parasitoid and a standard host under known conditions may provide material for comparative studies when the parameters are changed. These results may also be used to develop ecological models under laboratory or field conditions (Podoler and Mendel 1979).

The developmental stages of cynipid parasitoids have been studied in a relatively few species (James 1928, Havilan 1921, Simmonds 1952, Keilin et de la Baume-Pluvinel 1913). Several species of Pseudeucoila have been the object of morphological and physiological studies over several decades (Jenni 1951, Nappi and Streams 1969, Lenteren 1976a, Veerkamp 1980). Trybliographa rapae (Westood) is apparently the only species that has been studied within the genus. The life cycle and basic biology was described by Wishart and Monteith (1954). The purpose of this study was to describe the immature stages of T. daci in 2nd instar A. suspensa

larvae, and observe the main phenomena occurring in the host and parasitoid during the development from oviposition in the host to adult emergence. This study was considered necessary for future research and a better understanding of the relations of T. daci with the host.

Materials and Methods

Insects

Anastrepha suspensa colony. A. suspensa larvae were reared at 27.5° + 2°C, 50-60% of RH and a photoperiod of 14:10 L.D. in sugarcane bagasse base diet developed by R.M. Baranowski (unpublished) following the procedures outlined by Burditt et al (1974) as a host for T. daci. The initial source of A. suspensa was from a laboratory colony maintained at the Agricultural Research and Education Center in Homestead, Florida, but some later came from a subcolony maintained at the University of Florida Zoology Department in Gainesville.

Trybliographa daci colony. T. daci were allowed to parasitize A. suspensa larvae in two different types of parasitization units according to the host age. Small host larvae, 1-3 days old, were transferred from the bagasse diet to a plastic petri dish provided with a 2 mm layer of agar-base medium prepared by adding agar and sugar into boiling water in a proportion of 4:4:100. These oviposition units were refrigerated until 1 hr before being used. After parasitization the larvae were transferred with a spatula to fresh bagasse medium for maturation. Mature larvae were allowed to fall into moist vermiculite through a 4 mm mesh screen and reared in plastic boxes (20 x 12.5 x 8 cm) in a light and temperature controlled cabinet at 27-28°C and 14 hr light.

Attempts were made to expose small larvae inside the bagasse medium to parasitoids, but the percentage of parasitization was low. One difficulty in this procedure was the calculation of host-parasitoid ratio and time of parasitization.

Larger larvae (4-6 days old) in all cases were parasitized in "sting rings" described by Greany et al. (1976) for the mass-rearing of B. longicaudatus. The diameter of the embroidery hoop and the number of larvae varied. In order to maintain a degree of variability in the colony, host larvae of all ages were presented to the parasitoids. Both methods were used in the experiments according to the desired objective. In all cases, for general colony procedures or experimental purposes, parasitization was set up at room conditions (25°C, 50-60% RH, 12L:12D).

The emerging parasitoids were maintained in plexiglass cages (20 x 20 x 20 cm). These cages had a "sleeved entrance," and one screen side for ventilation. Parasitoids were fed honey provided in absorbent pads stuck to the walls; fresh water was supplied in glass vials with dental wicks. The parasitoids separated for experimental purposes were held in 20 dr plastic vials with a screened circular window in the base. A 10% honey solution was provided on a piece of cotton through a circular hole in the cap. Generally these insects were maintained at 20°C, and 14 hr light.

Developmental Studies

Second instar A. suspensa larvae 24 hr old were used to study the life cycle and development of the immature stages of T. daci. The host

larvae were presented to the parasitoid in agar units (9 cm diameter) containing 100 host larvae and 3 female parasitoids for 6 hr. This time of exposure was determined to give approximately 60% parasitism and very low percentage of superparasitism. A total of 12 oviposition units were used in the same way. After parasitization the host larvae and pupae were manipulated in the way explained earlier.

In order to observe the parasitoid development, 50 parasitized hosts were dissected every 12 hr, starting 6 hr after parasitization, until 3rd instar parasitoid larvae were found, and then every 24 hr until emergence of adult parasitoids. The hosts were dissected in vivo in a drop of physiological saline and observed under a dissecting microscope. The parasitoid egg, the 1st instar, and the smaller 2nd instar larvae were observed on the same microscopic slide with a phase microscope provided with an ocular micrometer. A semipermanent mounting of the egg and 1st instar in Hoyer's medium were used for photography.

Older parasitoid larvae were observed and measured under the dissecting microscope. Some specimens were cleared by immersion in chloral-phenol (25 parts of chloral hydrate + 30 parts of phenol) and a few drops of glacial acetic acid for several days. The gut of these large larvae was removed with forceps after puncturing the caudal end with an entomological pin. After clearing, larvae were mounted on Hoyer's medium and allowed to dry on a hot plate until ready for observation of sclerotized structures. When it was considered necessary for accurate observations, additional samples were dissected within the intervals established.

The number of each parasitoid instar was counted each time to calculate the proportion for each instar or stage at that time. Only parasitoids laid individually (1 per host) and not being encapsulated were counted. Observations of morphological characteristics, or movements of the living insects, as well as the nature of the hemocytic reaction of the host or changes in the host induced by the parasitization effect were noted. Each age interval varies ± 6 hr, which corresponds to the period of exposure to the parasitoid. Data are valid only for the age of the host and for the experimental conditions used during this experiment.

Results

Developmental Stages of T. daci

The duration of the immature stages was variable and a very approximate distribution of the rate of development and percentage of the stages found at different times are presented in Figure 1. The parasitoid has 4 larval instars, a short duration prepupal stage, and pupal stage.

Egg. The egg is stalked, monoembryonic and surrounded by an elastic chorion. The average increases in the size of the egg after laying and of first instar larvae are presented in Table 1. Figure 2 illustrates developmental changes in the eggs of T. daci in the host larvae following oviposition.

Twelve hours after oviposition the nucleus fills 45% of the cytoplasmatic space and increases up to 60% at the end of the 24 hr (Figures 2a, 2b). The segmentation of the embryo is evident at 38 hr (Figure 2c) and it is completed between 48 and 67 hr (Figure 2d); at that time all

Figure 1. Distribution of T. daci immature stages during development in A. suspensa 2nd instar host larvae, reared at 27.5 ± 2°C, 50-70% RH and 14 hr light.

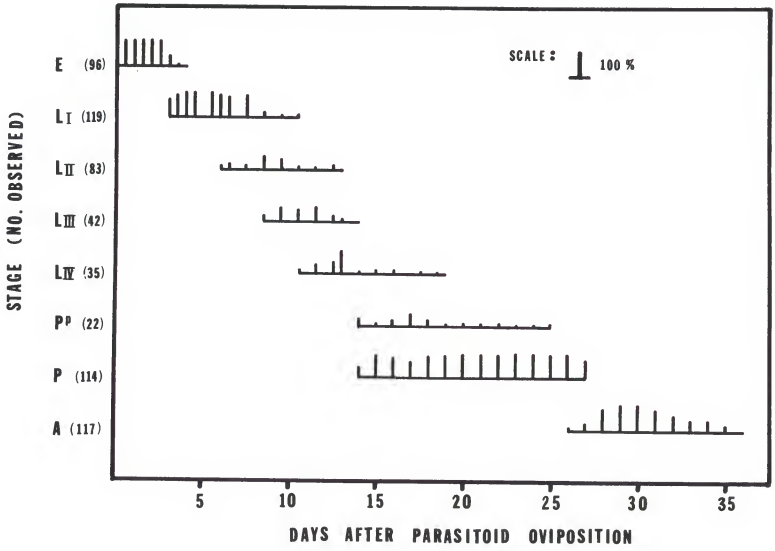

E (96)
LI (119)
LII (83)
LIII (42)
LIV (35)
PP (22)
P (114)
A (117)
SCALE : 100 %
STAGE (NO. OBSERVED)
5
10
15
20
25
30
35
DAYS AFTER PARASITOID OVIPOSITION

TABLE 1. Length ($\bar{x}$ µm ± S.E.) of T. daci eggs and 1st instar larvae reared in A. suspensa 2nd instar larvae at 27.5 ± 2°C, 50-70% RH 14:10 L.D.

Stage	Age (hr)	n	Length
Egg	Egg dissected from ovary	25	286 ± 0.01
	0 - 6	8	306 ± 5.17
	12 - 18	7	464 ± 11.90
	24 - 30	10	479 ± 14.78
	38 - 44	7	588 ± 11.50
	48 - 54	20	642 ± 13.88
	62 - 68	5	760 ± 16.73
First Instar Larva	0 - 6	14	957 ± 14.38
	12 - 18	14	967 ± 15.95
	24 - 30	14	1017 ± 15.93
	33 - 39	7	1135 ± 16.64
	56 - 62	14	1098 ± 34.39

Figure 2. Development of T. daci eggs in A. suspensa 2nd instar host larvae, at 27.5 ± 1 C, 50-70% RH and 14 hr light. Eggs dissected after parasitoid oviposition at 12 hr (a), 24 hr (b), 36 hr (c) and 68 hr (d).

a.
100μ
b.
100μ
c.
100μ
d.
100μ

the structures of the 1st instar larvae can be easily distinguished. When ready to hatch, at 70 hr, the larvae produce spinning movements.

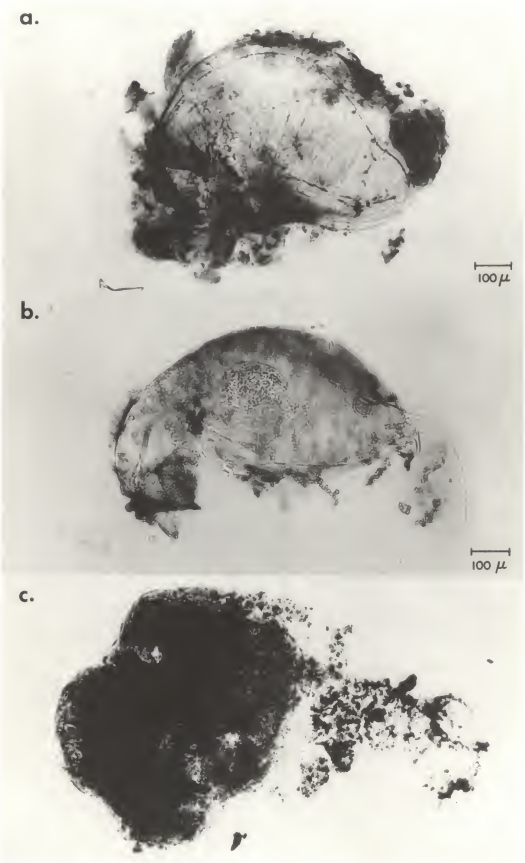
Trybliographa daci is encapsulated by the host hemocytes. The 1st encapsulated and partially melanized parasitoid eggs were observed 72 hr after parasitization, and this time was independent of the age of the host at the time of parasitization. At this time the egg had completed its development and in many cases it was possible to observe the 1st instar larva inside (Figure 3a). The egg was surrounded by a layer with melanotic areas irregularly distributed outside the chorion.

Eclosion. The eclosion of the first 30% of the eggs was observed at 73 hr after parasitization and was completed in the following 12 hr. The embryonic larva produces strong movements of the head and caudal end and breaks the chorion, which is retained around the body for a few hours and later is found in the hemocoel. Ecolosion is relatively uniform for eggs not affected by the encapsulation reaction. Only normally developing parasitoid stages were counted in this study.

First Instar. Larval instars are illustrated in Figures 4 and 5. Measurements of the 2nd, 3rd and 4th larval instars and pupae are presented in Table 2. The 1st instar larva is eucoliform type (Figure 4a) (Clausen 1940) and has a caudal appendage (Figure 4b) bearing numerous setae.

Second Instar. Second instar parasitoid larvae (Figure 5a) were observed from the 6th to the 12th day after parasitization, but a large percentage overlapped development of the 1st and 3rd instars. The second instar larvae are laterally depressed with differentiated head and 13 body segments. When newly emerged it bears a small tail, and the inner

Figure 3. Encapsulated T. daci by A. suspensa host larva at 72 hr (a), 86 hr (b) and 3 days (c) after parasitoid oviposition.


a.
100 μ
b.
100 μ
c.

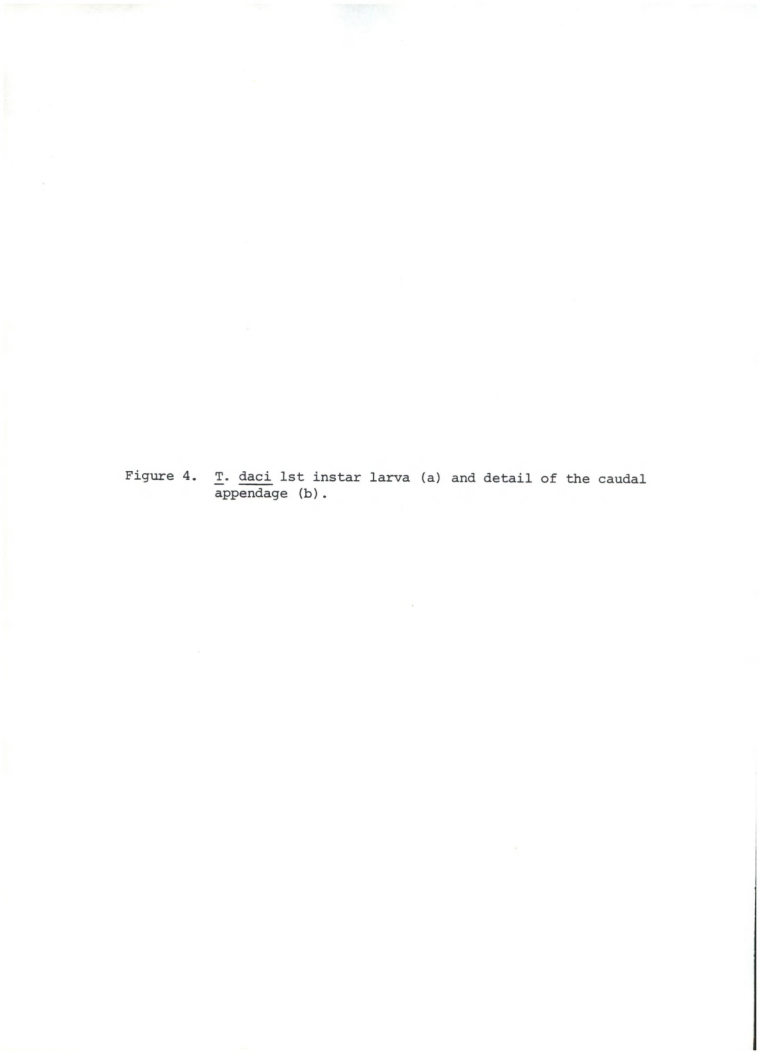


Figure 4. T. daci 1st instar larva (a) and detail of the caudal appendage (b).

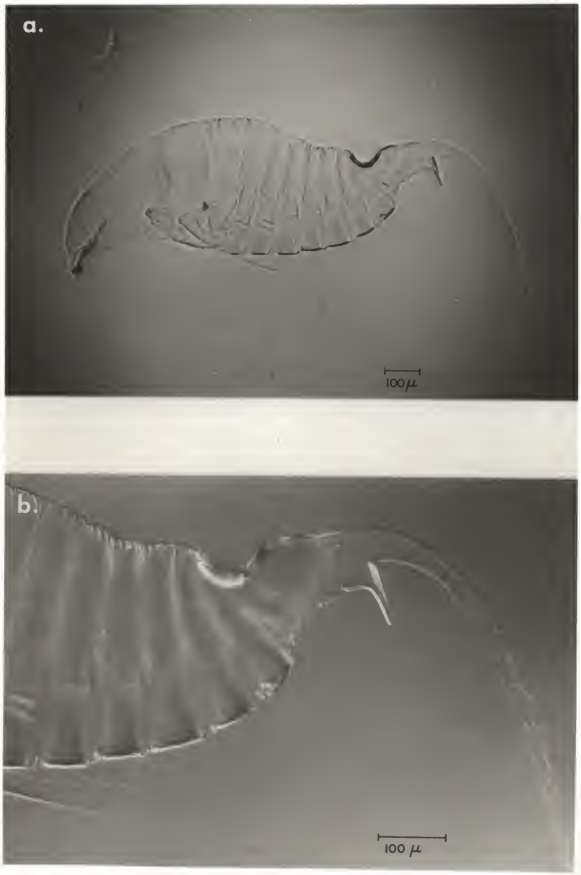
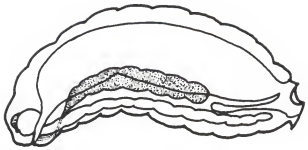
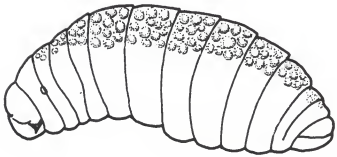

a.
100μ
b.
100 μ

Figure 5. T. daci, 2nd (a), 3rd (b), and 4th (c) instar larva.

a



b



c

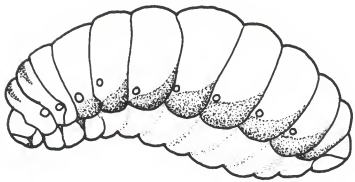


TABLE 2. Length and width ($\bar{x}$ mm $\pm$ S.E.) of T. daci larval instars, and pupae reared in A. suspensa 2nd instar larvae at 27.5 $\pm$ 2°C, 50-70% RH, 14:10L.

Stage	Age (hr)	n	Length	Width
II Larvae	0 - 6	7	0.815 $\pm$ 0.17	0.354 $\pm$ 0.022
	12 - 18	14	1.082 $\pm$ 0.07	0.387 $\pm$ 0.041
	24 - 30	13	1.213 $\pm$ 0.09	0.490 $\pm$ 0.028
	36 - 42	13	1.363 $\pm$ 0.15	0.576 $\pm$ 0.071
	48 - 54	5	1.264 $\pm$ 0.11	0.504 $\pm$ 0.038
	72 - 78	7	1.068 $\pm$ 0.09	0.498 $\pm$ 0.033
III	0 - 6	4	2.37 $\pm$ 0.27	1.04 $\pm$ 0.090
	15 - 21	8	2.52 $\pm$ 0.18	0.95 $\pm$ 0.053
	32 - 38	10	2.35 $\pm$ 0.09	0.86 $\pm$ 0.045
	48 - 54	8	2.60 $\pm$ 0.10	0.98 + 0.066
IV		20	2.93 $\pm$ 0.10	1.49 $\pm$ 0.039
Pupae ♂	72 - 76	19	3.02 $\pm$ 0.05	1.45 $\pm$ 0.039
♀	72 - 76	20	4.05 $\pm$ 0.03	1.51 $\pm$ 0.023

TABLE 3. Average length ($\bar{x}$ µm ± S.E.) of T. daci mandibles in II, III, and IV instars.

Instar	n	Length	Range
II	28	41.5 ± 1.85	85 - 50
III	20	80.65 ± 1.54	60 - 90
IV	20	110.75 ± 2.24	85 - 115

structures are visible through the transparent integument. The salivary glands lie latero-ventrally on the sides of the midgut. The posterior gut ends in a wide anus. Two short Malpighian tubules are directed forward. In older 2nd instar larvae the integument thickens, and becomes opaque and the larvae are larger. Measurements on mandibles of 2nd, 3rd and 4th instar parasitoid larvae are presented in Table 3. I found no differences in the length of the mandibles between large and small size larvae in the 2nd instar.

Third Instar. The 3rd instar (Figure 5b) has the characteristic "C" shape of hymenopterous larvae (Clausen 1940), with a distinct head and 13 body segments. The buccal opening is situated anteriorly and the sclerotized mandibles are visible with a dissecting microscope and good direct lighting. Only newly emerged 3rd instars are found inside the host; older 3rd instar larvae (>12 hr post molt) make a hole through the host tissues toward the dorsal wall of the thorax and come to lie between the host puparium and the host body, with the head directed toward the hosts' head. Some 3rd instars were observed at the 8th day of parasite age, but the majority were found from 9 to 11 days after parasitization. Due to the short duration of the 3rd instar, it has not been considered as a separate instar in other cynipids (James 1928, Havilan 1921).

Fourth Larval Instar and Prepupal Stage. These 2 stages (Figure 5c) are considered together because they are morphologically identical. The molt to the 4th instar takes place around the eleventh day, but the maximum number of parasites in this instar is observed during days 12 to 13 of the parasite cycle.

This larval instar is more representative of the hymenopteroid type of larvae. The head structures are poorly sclerotized. Only a pair of bidentate mandibles is detected with the dissecting microscope, but 2 pairs of sclerites located in the angular bases of the mandibles are observed in mounted specimens. These correspond to the anterior and posterior pleurostomal processes (Short 1952). The respiratory system is a modification of the holopneustic type (Imms 1951) with a pair of circular spiracles located in the meso- and metathorax and in each of the first 7 abdominal segments.

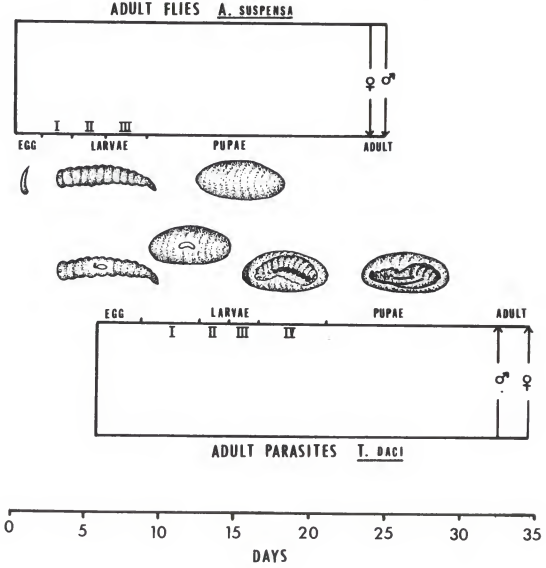
Pupae. The pupae are exarate, in which the appendages are loosely appressed to the body. Males are easily differentiated by their long antennae and smaller size compared to females (Table 2). The process of darkening starts a few days later and evidently males start the melanization 2-3 days before females. The pupal stage lasts approximately 13 days. The pupal meconium is partially excreted inside the puparium but may also be found outside or on the walls of containers. This meconium is a milky secretion that solidified almost immediately.

Discussion

Development of T. daci

In general the morphology of the immature stages of T. daci is very similar to the stages of T. rapae described by Wishart and Monteith (1954). Unfortunately these authors provide no data on the duration of the stages that would be useful as a point of comparison. Development of host and parasitoid based on the data for this experiment is diagrammatically represented in Figure 6. The development of hosts that escaped

Figure 6. Comparative development cycle of T. daci (parasitoid) and A. suspensa (host) reared at 27.5 ± 1C, 50-70% RH and 14 hr light.


ADULT FLIES A. SUSPENSA
♀ ♂
I II III
EGG
LARVAE
PUPAE
ADULT
EGG
LARVAE
PUPAE
ADULT
I II III IV
♂ ♀
ADULT PARASITES T. DACI
0 5 10 15 20 25 30 35
DAYS

from the parasite or that contained encapsulated parasitoids was not changed notably. Apparently there was an acceleration at pupation time compared with controls not exposed to the parasite but manipulated in a similar way.

First instar larvae are able to produce movements characteristically observed at eclosion, and may escape from the encapsulation mass and continue development. The disposed chorion with melanotic points is found in the host hemocoel. Parasitoids able to overcome the process usually remained as 1st instar larvae 2 to 3 days after non-encapsulated parasitoids had molted to 2nd instar. This phenomenon was also observed and described by Walker (1959) in P. bochei and was considered as "active" evasion of the parasitoid against the defensive reaction of the host. The active movements of the caudal end suggest an adaptative function to avoid the hemocytes.

The majority of parasitoids encapsulated did not overcome the encapsulation, and they were observed in dissected host larvae and pupae. The capsule generally became thicker and had a regular shape. Sometimes it included portions of Malpighian tubules or trachea from the host. Always the capsules contained inside a shriveled 1st instar larva that was still distinguishable when the capsules were dissected from host pupae or adult flies.

The external appearance and characteristics of the capsule formed against T. daci were very similar to those studied in P. mellipes reared in D. melanogaster. The main difference between the encapsulation of T. daci and P. mellipes was the time at which the process of encapsulation culminated. The first P. mellipes eggs surrounded with hemocytes

and partially pigmented were observed 33 hr after infection of the host (Nappi and Streams 1969). Histological examination of the capsule and the changes in the host hemocytes after parasitization in this last study showed precocious transformation of certain types of blood cells into lamellocytes, which finally are the main elements of the capsule. This capsule is classified as "sheath" type by Salt (1963) and Radcliffe and Rowley (1979). The differences in time of observation of encapsulation of T. daci and P. mellipes eggs are explicable on the basis of species differences and possibly the effect of rearing conditions, but the stage of development of the egg at the time of encapsulation in both species was the same. The duration of the egg stage in P. mellipes is 34 hr (Nappi and Streams 1969), and that of T. daci is 72 hr.

The Egg. Eggs were randomly laid in the hemocoel of the host body, but most of them were found in the abdomen and caudal end. The egg in the ovary had a petiole approximately equal to the body length, and in general, had the same appearance of eggs found in other cynipids (Clausen 1940, Hagen 1964). The measurements of egg and pedicel indicated a constant increase in egg size. The pedicel was maintained constant until the segmentation of the embryo was evident.

The duration of the egg stage in parasitoids is extremely variable and depends on the host and the specific parasite. In P. bochei, for instance, the incubation period was 48 hr at 23°C (Jenni 1951), and there was a difference of 3 hr between male and female eggs (Eijsacker and Bakker 1971a). In a similar species, P. mellipes, reared at 23°C the egg stage lasted 70 hr (Streams and Greenberg 1969).

An increase in size is a very common phenomenon in very small and almost yolkless eggs of many parasitic Hymenoptera (Fisher 1971), and the enlargement is due to the absorption of hemolymph and ions through membranes. The egg in T. daci increased 3 times in length from oviposition to complete embryonic development. Flanders (1942) classified the eggs of parasitic Hymenoptera on the basis of the utilization of the embryonic membranes to obtain nutrients from the host's fluids and described hydropic eggs as those in which the membrane becomes xenotrophic in function. The growth of the trophic membrane causes the egg to increase in size. Flanders (1942) considered one cynipid species, Ibalia leucospoides Hoch, to have hydropic eggs. This phenomenon was observed in other cynipids (Jenni 1951, Wishart and Monteith 1954, Keilin et de la Baume-Pluvinel 1913), but causative mechanisms were not investigated.

First Instar Larva. T. daci displays hypermetamorphosis, a phenomenon common to all cynipids. The characteristics of the lst instar larva are a latero-depressed head, thorax with fleshy processes and a tail from the last abdominal segment. The position usually adopted in mounted specimens made it impossible to determine the presence and characteristics of the mandibles. Around the buccal opening there are sclerotized projections, very mobile in living larvae, which could increase or reinforce the activity of the mandibles. Wishart and Monteith (1954) found a pair of short but defined mandibles in T. rapae but they were not found in the same species by James (1928). Keilin et de la Baume-Pluvinel (1913) did not find evidence of mandibles in Eucoila keilini Kieffer. The possession of opposable mandibles in primary larvae of hymenopterous parasitoidsis more associated with intraspecific competition with supernumerary parasitoids than

than with the feeding process (Fisher 1971). A pair of small projections were observed in the ventral side of the head of T. daci first instar larva; these are probably analogous structures to the prominent sensory papilla described in T. rapae.

Each thoracic segment bears a pair of non-segmented appendages used to dispose of the chorion. Apparently they are not used for locomotion. Living larvae moved in saline solution for a maximum of 30 minutes, but they did not show displacement in any direction.

The abdomen has 10 segments. In the dorsal part of the intersegmental membrane between the 7th and 8th segments there is a circular and very sclerotized aperture, which corresponds to the anus. There is no clear segmentation between the last 2 segments and both take part in the structure of the cauda. In the ventral aspect of the eight segments there are scale-like structures that are common also in other cynipids. These probably help in locomotion within the host tissue. The caudal appendage has been associated with respiration in other cynipids (Clausen 1940, Hagen 1964). The tail and other caudal structures are more likely to be related to evasion of host defenses as suggested before in this chapter.

The larvae are apneustic and probably acquire food and oxygen through the integument from the host fluids. This type of respiration and nutrition has been suggested in other cynipids and in many other hymenopterous parasitoids. Havilan (1921) supposed the use of the anus in Charipis sp. as a respiratory organ, since the heavily sclerotized cuticle did not appear adapted for gas diffusion. It is reasonable to assume the same type of respiration in T. daci.

The body of the 1st instar increased in length with age (Table 1), but the enlargement was entirely restricted to the thorax and abdomen. The head capsule was not shed.

First instar larvae were observed from the 3rd to 9th day after parasitization (Figure 1), when the host was still a 3rd instar larva. Some 1st instar parasitoids were present as late as 4 days after pupation of the host. Simultaneously a small percentage of parasitoids able to overcome the encapsulation elicited by the host were present, but they could be differentiated because the chorion with melanotic spots was found also in the host. Delayed development of the 1st instar was more evident in superparasitized hosts. Delayed development of normal T. daci 1st instar suggests a possible parasitoid-host hormonal dependence for parasitoid molting to the 2nd instar and initiation of feeding. Corbet, cited by Riddiford (1975), observed prolongation of the 1st instar of N. canescens until the end of the last instar of its host E. kuhniella. Feeding activity and development of the parasitoid were initiated when the host entered the wandering stage. Corbet associated parasite molting to the 2nd instar with changes in the hemolymph osmolidity. This phenomenon is related to the first release of prothoracicotropic hormone (PTTH) and ecdysone by the host in some lepidopterous hosts and, according to Riddiford (1975), the same physiological process occurrs in Ephestia.

Second and Third Instar Larvae. The earliest 2nd instar larvae were always found in host pupae, and never in host larvae. This is also basis for thinking that a host-physiological dependence for parasite

development exists. In superparasitized hosts both 1st and 2nd instars were found until 14 days after parasitization, but these cases were not considered to determine the distribution of immature stages presented graphically in figure 1.

There was no evidence of damage in the host tissues due to the parasite's feeding because the host was in a state of histolysis. Even so, the parasitoid probably was feeding because the gut showed semi-solid contents.

In the 3rd instar there is only one pair of circular spiracles, without a closing apparatus, located in the anterior border of the mesothorax. This type of respiratory system is a propneustic type (Imms 1951) and evidently facilitates the new aerobic respiration resulting from the parasite's escape from the host hemocoel.

Fourth Instar Larvae. At the end of the 14th day the 4th instar parasitoid had totally consumed the host and the larval meconium was found in the posterior end of the host puparium. The voidance of the larval meconium was considered as the end of larval development. The prepupal stage lasted 1 or 2 days, but very frequently the parasite remained in that stage for 15 days or more. The size and shape was very similar to the fourth larval instar and no molting process was observed between these two. The length of the mandibles (Table 3) is a reliable method for separation of the instars, especially when there are no obvious morphological differences. Measuring of the length and width of the body (Table 2) is not reliable as a guide to instar recognition, but gives information on the changes during the development.

Pupal Stage. The initiation of the pupal stage was demarcated by the development of the ommatidia of the compound eye under the 4th larval instar cuticle, followed by the constriction of the body separating the thorax from the abdomen, and differentiation of thoracic legs and wing pads. Complete pupae were observed at the 15th day of parasite age, after the last larval instar exuvia had been cast off. There was morphological differentiation between males and females, but the process of molting to this stage was simultaneous. When the parasite was ready to emerge, it removed the pupal skin using the mandibles and legs, and the active adult started opening an irregular emergence hole in the anterior part of the puparium.

Emergence of Adults. Only one parasitoid emerged from each host pupa. Not all parasitoids from the same period of egg deposition developed and emerged as adults at the same time. Under the conditions of this study most of the males emerged at 26-27 days of age, and the majority of females did so at 28 days. There was a remarkable asynchrony in the emergence and some females and males emerged during 10 days after the maximum peak was observed.

The time of development of a parasitoid is influenced by the host and parasitoid species, by the age and nutritional suitability of the host and by environmental conditions (Salt 1941, Legner 1969, Vinson 1980). Parasitoid development is slowed down when several parasitoids are present on the same host (Corbet and Rotheram 1965). Information on the rate of development and rhythm of emergence of T. daci in other hosts is scanty. The development rate of the strain used in this experiment was 23 days at 25°C and 80% RH, when reared in 2nd instar larvae of C.

capitata (Pralavorio 1980, personal communication). Ahmad et al. (1972) reported 21 to 28 days for the same species reared in D. zonatus at 24 ± 3°C. The differences in all 3 cases may result from the host species.

Asynchrony in emergence is advantageous to a parasitoid under field conditions because the emerging progeny will be present over a long period. The parasitoid will be in contact with a larger number of hosts as they develop to stages suitable for parasitism. Such asynchrony also assures a maximum parasitoid level which should be advantageous since it increases genetic variability. The A. suspensa-T. daci host:parasitoid relationship seems to conform closely to this system (R.E. Sailer, personal communication).

CHAPTER III

COURTSHIP, MATING, OVIPOSITION AND MODE OF REPRODUCTION OF TRYBLIOGRAPHA DACI

The free-living female parasitoid is of special concern to entomologist engaged in biological control. The behavior of the mature female is commonly the major determinant of the efficiency of the species as a controlling agent of its host (Doutt 1964, Doutt et al. 1976). Certain behavioral patterns such as mating, oviposition, longevity, and nutrition are difficult to observe under field conditions. These aspects have to be studied under controlled laboratory conditions.

Mating and oviposition habits are in general similar in parasitic hymenoptera, but the type of reproduction varies within the same taxon (Doutt 1964). Most parasitic hymenoptera exhibit facultative parthenogenesis, i.e., the egg may develop with or without fertilization. In these cases, fertilized eggs are diploid and give rise to females, and the unfertilized eggs are haploid and give rise to males. This haplodiploid system is called arrhenotoky (Doutt 1964, Chapman 1971).

T. daci adults exhibit sexual dimorphism. Males can be recognized by their long antennae, while females have short antennae. Females emerge at least 24 hours after the males. The courtship and mating behavior and type of reproduction have not been described for T. daci.

The following series of experiments had as main objectives description of the courtship, mating, oviposition, and determination of the sex ratio of the surviving progeny of mated and virgin female T. daci.

Materials and Methods

Courtship and Mating

Unmated females and naive males were obtained from parasitized host pupae kept individually in small glass vials. After emergence they were left in the same vial at room condition and fed honey solution. To observe the courtship and mating behavior virgin females 3-, 6-, 18-, 24 hr and 2- and 3-days old were isolated with a male partner in small petri dishes (3 cm x 1 cm) and observed under a dissecting microscope until mating or attempted mating occurred. The females were isolated after that encounter in individual vials until the next day, when they were again observed in the mating dish with the same male or with a new naive male. Males were of different age with respect to the female. Each age was replicated 5 times. When courtship was not exhibited within the first 30 minutes, the observation was concluded and the female was isolated for new observation within the next 3 or 4 hours.

Oviposition

The oviposition of T. daci females was observed by using 3rd or 2nd instar A. suspensa as a host. Larvae were presented individually to females taken from the colony cages. T. daci females were presented with

individual 3rd instar host larva pressed between 2 pieces of cloth and positioned under the center of a plastic lid. This oviposition unit was covered with a plastic petri dish base under which the adult female was enclosed. Small host larvae, 1st or 2nd instar, were presented in small petri dishes 3.5 cm diameter containing agar based medium. In both cases oviposition was observed under a dissecting microscope. The differences between oviposition and probing were not established. A subjectively determined longer oviposition was taken as actual oviposition and short "stings" in the host were considered as probes. The number of "stings" with the ovipositor in the host larvae was counted, and the larvae were dissected 24 hr later to determine the number of parasitoid eggs.

Determination of Arrhenotoky

Six virgin females, 12 hr old, were placed individually in small vials with a male until mating was observed. Each female was supplied daily with 30-40 2nd instar larvae in a 9 cm diameter petri dish during 6 hr. New larvae were provided during 3 consecutive days. The parasitized larvae and pupae were maintained at 27°C, 60% RH, 14L:10D until parasitoid emergence. The same experimental procedure was followed with mated females. During the intervals of no oviposition females were kept in marked and separate vials and fed honey solution via a piece of cotton.

Results

Courtship and Mating

T. daci males exhibited precopulatory behavior only in the presence of females older than 6 hr, but younger than 2 days. Experienced or

inexperienced males did not display premating movements in the presence of females younger than 3 hr or those 2 or 3-days old, at least during 30 minutes of observation. The initiation of courtship by males was delayed for up to 15 min in the presence of virgin females 6 hr old, but started after a few seconds when the females were 18 to 24 hr old.

Courtship started with movements of the male in a straight line or in circles toward the female. These movements were simultaneous with fast vibration of the wings. Wing beating was interrupted during the periods at which the female was touched by the male's antennae. This courtship period was very variable and lasted 7.5 $\pm$ 1.7 min ($\bar{x} \pm$ SE) (n = 17) when the females were 5 hr old, and 49.1 $\pm$ 7.5 sec (n = 17) when the females were 18 to 24 hr old.

After being touched with the males' antennae the female became static and was mounted by the courting male. Once mounting had occurred, the speed of the male's wing beating increased and simultaneously the male's antennae were moved in circles until the female's antennae were touched. The receptive female responded by bringing her antennae from a vertical position to a backwards and horizontal position. At this movement the male bent his abdomen toward the female genitalia and his body was vertical in relation to the female's body. These premating movements lasted 8.77 $\pm$ 1 sec (n = 18). A female ready for copulation exposed the genitalia and mating occurred. Mating lasted 7.8 $\pm$ 0.7 sec (n = 18). After mating and dismounting by the male, the female preened her abdomen with the metathoracic legs and spent some time preening the antennae and mouth parts.

It is important to note that 2 females presented to experienced or inexperienced males in succession from 3 hr to 2 days were not mated; however, they were approached and mounted several times. Mated females were not mated for a second time but they were courted. Mated females did not actively reject a male except immediately after being mated. In this latter case she ran away from the approaching male. Males were able to mate multiple females, but the data are not sufficient to indicate the maximum capacity of male mating.

I frequently observed simultaneous mounting of a female by 3 or 4 males in the colony cages. Those males attempted to reach simultaneously the female genitalia. Another phenomenon was the display of a complete courtship toward female cadavers left in the cages. Wing vibration and circular movements were observed in males released in cages previously occupied by females despite the females absence. These observations may indicate the involvement of odors, perhaps a pheromone, in courtship and mating.

Oviposition

When the female was confined with a host, she spent time walking on the walls and upper part of the cover for up to 2 hr. Upon discovery of the host in the agar medium, she probed with the ovipositor several times in the medium around the host, and touched the host with the tarsus. The antennae were moved constantly up and down, but apparently were not used to touch the host. The host larva moved actively from its original site after being pierced by the ovipositor of the parasitoid but after several seconds it became quiet. After oviposition or attempt

at oviposition in the host the female moved away from the host and back to continuous searching in the same area. The average ($\bar{x} \pm$ SE) number of "probes" per host larvae counted before I considered that an egg was laid was equal to 2.26 $\pm$ 0.56, and 4.66 $\pm$ 0.73, for 2nd and 3rd instar larvae, respectively, based on 15 larvae observed per age.

Determination of Arrhenotoky

The numbers of male and female progeny per mated and virgin T. daci female for each oviposition period are presented in Table 4. Virgin females gave rise only to male progeny, whereas mated females produced males and females. The average female:male ratio of the mated female progeny was equal to 1:1.8, 1.1:1 and 1:1.2, during the 1st, 2nd and 3rd day of oviposition.

Discussion

Courtship and Mating

The courtship behavior of T. daci is very similar to that observed in P. bochei by Assem (1969). The main differences from those results were the duration of mating and the time at which females became non-attractive to males. In P. bochei mating lasted 1 to 3 min and females remained attractive up to about 3 weeks, after which time they were not receptive even though occasionally courted.

The very short mating time in T. daci might result from the experimental procedure. Mating between parasitoids of the stock colony lasted up to 1 min, indicating that the larger space in the rearing cages could influence behavior. Veerkamp (1980) found significant differences in

Table 4. Progeny of virgin and mated T. daci females exposed to 30 to 40 2nd instar A. suspensa larvae for 6 hr each day.

Parasitoid age (days)	Female number	Host pupae recovered and progeny per female			
		Mated Females		Virgin females	
		Host pupae	♀/♂	Host pupae	♀/♂
1	1	30	8/11	40	0/27
	2	39	12/22	27	0/22
	3	19	6/8	32	0/22
	4	40	9/27	40	0/14
	5	33	4/9	29	0/13
	6	33	4/9	29	0/13
2	1	34	24/9	34	0/20
	2	32	10/13	36	0/26
	3	29	2/25	35	0/27
	4	31	0/0	40	0/0
	5	31	1/3	32	0/14
	6	25	4/1	20	0/0
3	1	25	13/0	29	0/18
	2	34	11/20	18	0/14
	3	28	2/12	30	0/1
	4	*	*	38	0/5
	5	*	*	35	0/24
	6	*	*	30	0/12

* The female parasitoid died.

the duration of the copulation between strains of P. bochei, and this characteristic behavior was genetically transmitted to the progeny. The duration of the copulation was not related to fertilization of eggs. The differences in sex ratio of the progeny were related to genetic differences between the strains.

The importance of pheromones in the mating behavior of other parasitoids has been reported. Schilenger and Hall (1961), for example, reported that males of Trioxys utilis Muesebeck, a parasitoid of the spotted alfalfa aphid, Theroaphis maculata (Buckton), detected virgin females by odor rather than by sight. Studies with Opius olleus Muesebeck, a parasitoid of the apple maggot, indicated the presence of a female secreted attractant (Boush and Berwald 1976). A volatile pheromone extracted from C. sonorensis females elicited courtship behavior from males (Vinson 1972a). The display of courtship by T. daci males toward female cadavers might indicate the mediation of mating through an attractive substance and/or the use of visual stimuli.

Lack of initiation of copulatory movements by T. daci males in the presence of 3-day old females was surprising. It might be related to the available space and the production of a pheromone. The decline of pheromone production due to the female age could explain the lack of male mating behavior. In short life span species like T. daci, pheromone production should start at an early age, and might also terminate early.

The presence of courted, but not mated, females of P. bochei was observed by Assem (1969), who called them pseudovirgins. The occurrence of these females might influence sex ratio. Thus, it would be erroneous

to assume that all T. daci females in a colony cage were mated. In sex ratio determinations actual observation of the mating pairs is necessary.

Copulation immediately after emergence has been reported in other cynipids, e.g., Hexacola sp. (Simmonds 1952), T. rapae (Wishart and Monteith 1954), but there is little detailed information relating to courtship except notation of wing vibration by courting males. Wing vibration is of particular interest, and has been interpreted by Vinson (1972a) as a mechanism to orient the males to a source of odor. Yoshida (1978) reported the secretion of a pheromone by Anisopteromalus calandrae (Howard) females that elicited wing vibration of males.

Oviposition

The number of probes by the ovipositing T. daci female was related to the size of the host. Relationship between the size of the host and the number of probes was observed also in P. bochei by Eijsacker and Lenteren (1970). They concluded that the probability of "hitting" the host depends on the proportion of the surface area of its body and the total surface area of all the hosts present. Movements of the host larvae after being pierced were observed also in P. bochei (Lenteren 1976a) and were considered to result from injection of a paralyzing poison before egg-laying.

Demonstration of Arrhenotoky

The production of males from virgin females proved that T. daci reproduces by arrhenotoky, a process in which males develop from unfertilized eggs. This process was associated with haploidy of males

(Hamilton 1967). Females were produced by mated parent females only.

Arrhenotoky was reported in other cynipids, e.g., T. rapae (Wishart and Monteith 1954), P. bochei (Eijsacker and Bakker 1971a), Charipis victrix Hartig (Havilan 1921). Apparently only Callaspidia defonscolombie Dahlbon reproduced by thelytoky (Rotheray 1979), in which case female progeny are produced from unfertilized eggs.

Females of T. daci were able to produce viable female and male offspring during the first day of life. Females had an average of 32 ovarioles (range 29-36, n = 13), and each ovariole had 3 or 4 developed eggs. Similar observations were reported in other cynipids. Female cynipids generally lay eggs at an early age. Pseudeucoila sp., for example, deposited 99% of the eggs in the first 5 days of oviposition. The eggs laid during the first day yielded 72% of the total female progeny (Chabora et al. 1979).

Prior to the observation that 1-day old female T. daci could produce viable progeny, I had intended to use 3 to 5-day old females in experiments for progeny production and colony maintenance. For mass production of parasitoids or releases in the field it may be advisable to use young females.

CHAPTER IV

EFFECT OF RELATIVE HUMIDITY AND TEMPERATURE ON DEVELOPMENT AND LIFE SPAN OF ADULT TRYBLIOGRAPHA DACI

The regulation of optimum relative humidity (RH) and temperature during the development of insects or in adult stages is of primary importance. The water content of insects varies from 50 to 70% of the body weight, including the cuticle, but the content of the internal tissues is higher. Reduction from a critical level leads to death (Chapman 1971). The mechanism of absorption and regulation of water is the same in small and large arthropods. For small arthropods it is more difficult to maintain the inner balance under fluctuating condition (Machin 1979). Parasitoids take water from the host fluids; thus, the requirements of RH for normal development may be expected to be within the same range for host and parasitoid.

There is, for each insect species, a fairly well defined range of temperatures within which the organism remains viable. The exact cause of death at the limits of the viable range has not been extensively studied. Changes at the molecular level and other effects, such as changes in metabolic balance, may play an important role (Bursell 1964).

Dissection of parasitized hosts from the stock colony and study of the life cycle of T. daci showed a proportion of dead parasitoids in pharate pupae or pharate adults. This effect may have resulted from low

or fluctuating relative humidity. These observations motivated the study of the effect of different but constant levels of RH on the development of T. daci and on the life span of adults.

Materials and Methods

Effect of Relative Humidity on Development of Parasitoids

Some instar A. suspensa larvae that were 24 hr old were presented for 6 hr to the parasitoid in petri dishes (9 cm diameter) provided with a layer of agar. Each dish contained 100 host larvae and 3 female parasitoids. The parasitoid females were 5 - 6 days old and they did not have oviposition experience. The control larvae were treated in a similar way except that they were not exposed to parasitoids. Newly formed host pupae, 150 per replicate, were placed in plastic containers (9.5 cm high, 7.5 cm diameter on the top and 4.5 cm diameter on the bottom) inside a 6 cm layer of moist (50% water by wt) vermiculate. These containers had 2 ventilation spaces (2.5 cm x 4 cm) on the sides covered with fine mesh screen.

Parasitized host pupae and controls were reared in 3 separate incubators at 27.5 $\pm$ 2°C, 14 hr photophase. Each cabinet was maintained at a different, but constant, level of RH until emergence of flies and parasites. The levels of RH (treatments) were 50, 60 and 70%. Each treatment had 3 replicates and 1 control. The number of flies and parasitoids that emerged and the number of hosts were noted per treatment and replicate. A sample of host puparia from which neither flies nor parasites emerged was dissected after the experiment was over to determine why no flies or parasitoids had emerged.

TABLE 5. Effect of 3 levels of relative humidity upon the development of T. daci and its host A. suspensa.

% RH	% T. daci emerged[1/]	% A. suspensa emerged[1]	% Intact A. suspensa puparia[1/]
70	68.44 ± 1.37a,[2]	10.44 ± 0.30a	21.10 ± 2.68a
60	57.33 ± 0.94b	14.88 ± 1.36a	27.77 ± 2.19a
50	35.77 ± 7.35c	12.44 ± 5.38a	51.55 ± 11.74b

1/ $\bar{x}$ ± SD of 3 rep./150 pupae each per RH level.

2/ Means in the same column followed by the same letter are not significantly different (P<0.05) as determined by Duncan's Multiple Range Test.

Influence of Temperature and Relative Humidity on Life Span of T. daci Adults

Groups of 50 male and 50 female T. daci that emerged from the stock colony were maintained in plexiglass cages (20 x 20 x 20 cm) in incubators at 14 hr photophase and at selected constant RH and temperature. The insects were fed honey and water. Relative humidities of 50, 60 and 70% at a constant temperature of 27.5 $\pm$ 2°C and temperatures of 20, 24 and 27 $\pm$ 2°C at 60-80% RH were evaluated.

The dead insects were taken out of the cages every 2 or 3 days and water and food were supplied on the same days. The average life span and percentage survival relative to the total number of individuals observed were determined from the mortality at each interval.

Results

Effect of Relative Humidity in the Development of T. daci

The effect of relative humidity upon the percentages of parasites and flies emerging is presented in Table 5. Successful development of parasitoids was proportional to relative humidity and developmental success was significantly different ($P < 0.05$) in each treatment. No significant difference was observed in the percent of flies that emerged from the parasitized host pupae. More host puparia died without giving rise to either adult parasitoids or adult flies at the lowest RH of 50% and this mean was significantly different from the mean host mortality in the other treatments.

The emergence of A. suspensa from the control groups was very similar (89.33%, 80.00% and 81.33% at 70%, 60% and 50% RH, respectively)

in the 3 treatments and fell within the range established by Ashley et al (1976). The difference between percentage of total survival (flies plus parasitoids) in the treatments and the survival in the corresponding controls was equal to 13.59%, 7.78% and 33.12%, at 70%, 60% and 50% RH, respectively. Higher mortality was observed at lower RH.

The dissection of puparia without emergence holes proved to be of little value because the contents were very necrotic. General evaluation indicated that the parasitoids were more affected in the pharate pupae or pharate adult stage. At 70% RH, 35% of 42 puparia observed contained healthy pupae in the process of darkening, 4.7% had adults ready to emerge, and 59% were necrotic. The proportion for the same stages at 60% RH showed the following results: 27% of 80 puparia dissected contained dead pharate adults, 25% 4th instar parasitoid larvae in development and 47% were necrotic and the content was not identified. A drastic effect upon pharate adults was observed at 50% RH which 48% of the 91 puparia dissected contained pharate dead adults, 23% contained dead larvae, and 29% were not identified.

The developmental time of T. daci males and females that emerged was very similar at the 3 RH levels. Among males, 68% emerged between 27-28 days after parasitization, while 75% of the females emerged between 28-29 days. The remaining 32% of the males and 25% of the females recorded emerged within an additional 10 days and then the experiment was terminated. The asynchronous patterns of emergence were the same as those observed in the general colony.

The sex ratio of parasitoid progeny favored females at all treatments. The female:male ratio was 1.9:1, 1.8:1 and 2.3:1 for 70, 60 and

50% RH, respectively. Surprisingly a larger number of pharate adult females than males were found dead inside the host puparium. The sex ratio may indicate the influence of the parent female in the determination of the progeny sex. The females used for this experiment had no prior experience in oviposition.

Influence of Temperature and Relative Humidity Upon the Life Span of T. daci Adults

The survival of males and females at the selected relative humidities was similar in all treaments (Figure 7). Females had similar life span at 60 and 70% RH but the males' life span was shortened at 60% RH. The RH of 50% reduced the life span of females but did not affect males (Table 6). The time elapsed to observe 50% of mortality was very close to the average life span for both sexes at all treatments, except for females maintained at 50% RH as shown in Table 6. At 50% RH 50% of females died on the seventh day of life.

There was an inverse relationship between temperature and life span (Table 6). The survival of males and females was similar at each selected temperature (Figure 8). Mean longevity of males and females was prolonged at $20^{o}C$ and reduced at the highest temperature of $29^{o}C$ (Table 6). The mean longevity of 23 days for adults maintained at $24^{o}C$ was longer than that observed at any RH level and a constant temperature of $27.5^{o}C$ (Figure 6).

Figure 7. Survivorship of T. daci males and females at different but constant relative humidities and temperature of 27.5 ± 2°C.

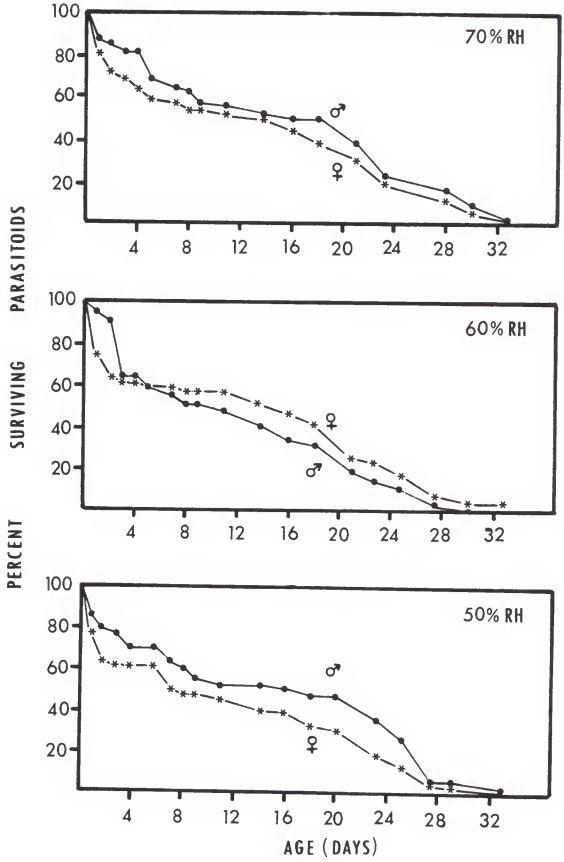

PERCENT SURVIVING PARASITOIDS
70% RH
60% RH
50% RH
♂
♀
100
80
60
40
20
4
8
12
16
20
24
28
32
AGE (DAYS)

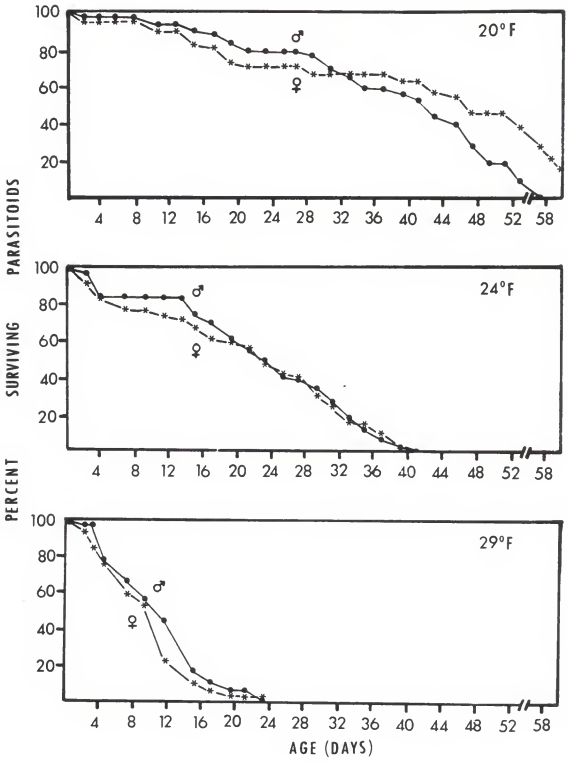
TABLE 6. Effect of relative humidity and temperature on longevity of T. daci adults.

	Longevity (days) X + S.E.		Time required for 50% mortality (days)	
	Males	Females	Males	Females
Relative Humidity[1]				
(%)				
50	14.8 ± 1.6	12.4 ± 1.4	16.6	7.3
60	12.3 ± 1.4	13.8 ± 1.6	10.0	16.4
70	14.7 ± 1.6	13.3 ± 1.6	14.3	14.3
Temperature[2]				
°C ± 1.0				
20	38.2 ± 2.5	41.6 ± 2.4	44.64	47.6
24	23.8 ± 1.6	21.4 ± 1.5	23.0	23.2
29	10.5 ± 1.0	9.5 ± 0.6	10.8	9.28

[1] Temperature 26.5 ± 2°C.

[2] Relative humidity 60-80%.

Figure 8. Survivorship of T. daci males and females at different constant temperatures and relative humidity of 60-70%.


PERCENT SURVIVING PARASITOIDS
100
80
60
40
20
♂
♀
20°F
24°F
29°F
4 8 12 16 20 24 28 32 36 40 44 48 52 58
AGE (DAYS)

Discussion

Influence of Relative Humidity on Development of Parasitoids

The RH requirements are generally high for parasitoids because they normally develop in an aqueous medium, i.e., the hosts' body. Results from experiments with varying RH indicate that high RH is critical for development of T. daci and that at least 70% RH with a moisture content of 50% (W:W) in the pupation medium should be maintained. T. daci can successfully develop within the range of RH and temperature previously established as most suitable for the host, A. suspensa (Prescot and Baranowski 1971, Ashley et al. 1976). The high mortality of pharate adults at low humidity indicated that the parasitoid was able to continue its development but was not able to bite its way out from the host puparium. Consequently a constant and high humidity must be maintained during the whole cycle.

Pralavorio (1980, personal communication) indicated need for a higher RH for T. daci when C. capitata larvae were hosts than for other species of fruit fly parasitoids reared in Antibes, France. The higher RH required may be related to migration of the 3rd instar from the host hemocoel to a drier habitat between the body of the host and host puparium. This behavior is not characteristic of other species.

Effect of Temperature and Relative Humidity on the Life Span of T. daci Adults

The effects of extrinsic factors such as temperature, relative humidity, nutrition and radiation determine a characteristic life span for insects (Clark and Rockstein 1964). Longevity in adult insects is

inversely proportional to the metabolic rate, high temperature increases the rate of heat production and oxygen consumption and accelerates the aging process (Clark and Rockstein 1964, Bursell 1964). The short life span of T. daci at 29°C is consistent with the expected higher metabolic rate at 29°C. Adults at this temperature were very active, while those at 20°C were inactive for longer periods. Longevity of T. utilis was prolonged to 25 days at 15°C and 80% RH but was reduced to 1 day at 24°C and 30% RH (Schlinger and Hall 1961).

The survival rate of T. daci under selected RH and temperatures in this experiment followed approximately the tendency of constant mortality per unit of time (Southwood 1978). These results do not mean that this curve is the specific survivorship curve for T. daci. To make a conclusion on the kind of curve for a particular species it is necessary to standardize data from different strains of the species under different experimental conditions (Deevey 1947).

Storage of experimental adult parasitoids at low temperatures has been the current method for extending longevity and the supply of parasitoids. Streams (1968), for example, maintained P. bochei females up to 100 days at 7°C. Refrigerated females that were 33 days old were equally fecund to non-refrigerated females that were 1-day-old, but storage for more than 100 days caused a reduction in fecundity. Storage of experimental T. daci at low temperatures would be advisable because of the short life span at high temperatures.

Based on the experimental data presented here, it would seem advisable to maintain the colony of T. daci at 24-26°C and at about 60% RH.

CHAPTER V

EFFECT OF ANASTREPHA SUSPENSA LARVAL AGE ON PREFERENCE, DEVELOPMENT AND CHARACTERISTICS OF TRYBLIOGRAPHA DACI PROGENY

One of the first steps in rearing a parasitoid is selection of a host age in which it can successfully develop. According to Salt (1938) a suitable host is one in which a parasitoid can generally reproduce fertile offspring. Thus, the host must meet physical and chemical requirements to be accepted as a site for oviposition, and then allow the successful development of the parasitoid in or on it.

Vinson (1975, 1980) considers the acceptance of a host for oviposition by the parasitoid as the last step in the process of host selection, and restricts the concept of host suitability to factors affecting development of the parasite. Sometimes the female may choose a host for attack, and even though oviposition occurs, the parasitoid progeny may be unable to develop if the potential host is immune or otherwise unsuitable (Salt 1938, Doutt 1964). After it has been located by the female parasitoid, the preference of a host is influenced by the physical and physiological state of the host. The degree of acceptance of a host for oviposition by the female of an internal solitary parasitoid depends on her discriminatory capacity to distinguish the suitability of the host (Lenteren 1976b, Fisher 1971, Vinson 1980).

Attraction to a host is not always an indication of acceptance for oviposition. Salt (1938) observed adults of Trichogramma sp. drilling

on eggs of Orgia antigua (L.) and Smerinthus populi L. but he did not find them parasitized. These host eggs were considered physically unsuitable because of their thick chorion. The analysis of the oviposition behavior of P. bochei in different ages and species of Drosophila larvae showed differences related to host body surface area, thickness of the cuticle and preference for a host in which the female parasitoid has itself developed (Eijsacker and Lenteren 1970). The choice of a specific host and age may be based upon the host hormonal stage (Fisher 1971, Riddiford 1975), or in response to chemical stimuli from the host or host products (Vinson 1976) acting as chemical messengers called kairomones (Brown et al. 1970). After oviposition the host constitutes the particular parasitoid environment with physical, chemical and physiological characteristics that are the determining factors for the offspring development.

According to Salt (1941) the host, instead of being a passive victim, presents a number of parameters that may affect parasitoid physiology or morphology. The rate of development of immature parasitoid stages in relation to host age has been studied closely in some cases, and the results show specific interrelationships in each host-parasitoid system (Fisher 1971). Some times the duration of the parasite development decreases with increasing age of the host at the time of parasitization (Miles and King 1975, Beland and King 1976, Beckage and Riddiford 1978), or alternatively, the parasitoid may develop faster in younger hosts (Coats 1976, Lawrence et al. 1976, Podoler and Mendel 1979). The effect may be due to nutrient supplies (Salt 1941) or to the number of parasitoids present within the same host (Beckage and Riddiford 1978). Other physiological effects can

be evaluated in the adult progeny through the number of eggs (fecundity), physical vigor, changes in behavior, and differences in sex ratio (Salt 1941). Morphological effects are usually reflected in the total size of the emerging parasitoids, or improportional appendages which finally interfere with its normal development (Salt 1938, Vinson 1980). The most drastic effect results from the host hemocytic reactions generally expressed by encapsulation. These reactions ultimately kill the parasitoid unless it can, by active or passive means, overcome host defenses (Salt 1963, Vinson and Iwantsch 1980).

The general procedure for the initial colonization of T. daci used 3rd instar A. suspensa larvae. Nevertheless the percentage of successful parasitism was very low. Presented here are the results from a series of tests utilizing different ages of the same host parasitized by 5 - 6 days old parasitoid females during 24 hr, with the objective to evalute the effect of host larval age on preference for oviposition, progeny, survival, development time, and morphological characteristics of the parasitoid adult.

Materials and Methods

T. daci adults obtained from the stock colony at 5-6 days of age (reared in 3rd instar A. suspensa larvae) were transferred from rearing cages after 24 hr oviposition experience to clean, wooden frame cages (49 x 19 x 30 cm) with a glass top. These parasitoid adults were allowed 6 hr to accommodate to their new cage, and then they were given A. suspensa larvae of different host ages for oviposition tests.

To obtain uniform host ages, fruit fly eggs were collected within 6 hr of oviposition, washed with 0.05% sodium benzoate and incubated

for 2 days. Then they were spread on sugarcane bagasse and reared at 27°C and 80% RH. The stage of development of host larvae was checked before exposure to the parasite by observation of the mandible (Lawrence 1979) of a small sample using a dissecting microscope.

Host Age Choice

A. suspensa larvae 1-, 3-, 5- and 6-days old corresponding to 1st, 2nd, young 3rd and old 3rd instars, respectively, were exposed simultaneously to 50 female parasitoids. At each age 100 host larvae were exposed in 9 cm diameter embroidery hoops, (sting units) containing a small amount of diet. Three sting units per age and controls with host-contaminated diet were arranged into a parasitization cage in 5 rows and 3 columns, with each column containing one host age sting unit and one control. The proportion, of the total number of females, observed on each age host or control at different intervals was used as an index of host age preference by the female parasitoid. After 24 hr of parasitization each age group and replicate were reared separately in the way explained in general procedures. One replicate at each age was dissected after host pupation to evaluate the number of parasitoids per host and the state of parasitoid development. The remaining replicates were reared until emergence of flies or parasitoids. The entire experiment was replicated 2 times. The parasitization time (24 hr) and conditions during the parasitization period (26°C, 50% RH and 14 hr photoperiod) and rearing condition of parasitized host (27.5°C, 80% RH and 14 hr light) were the same in both replicates.

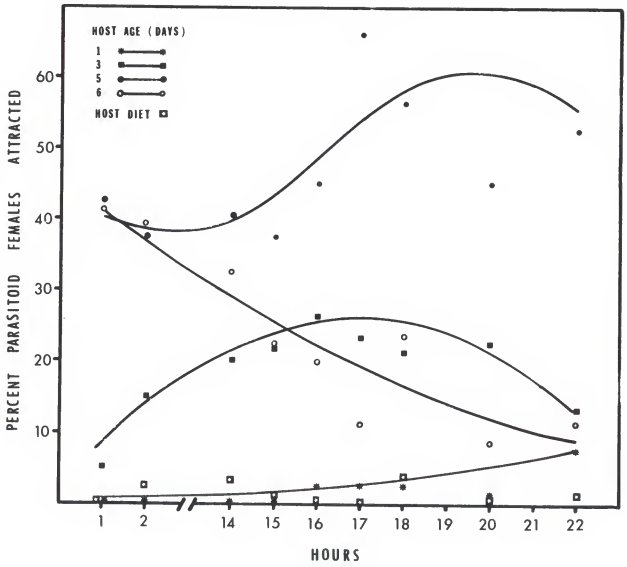
Single Host Age Parasitization Experiment

Eggs of A. suspensa were collected within 6 hr of oviposition and after hatching the larvae were removed from the medium every day for 6 days. The age of these host corresponded, respectively, to 1st, early 2nd, late 2nd, early 3rd and late 3rd instars. Larvae were exposed to T. daci using separate sting units for each age. The 1-, 2-, and 3-days-old larvae were parasitized in disposable (9 cm diameter) petri dishes with a layer of agar base medium. The host larvae 4-, 5-, and 6-days-old were exposed in embroidery hoops sting units (9 cm diameter) inclosed in a plastic box (12 x 17 x 6 cm) with screened lid. Each sting unit containing 100 larvae (3 replicates/age) was exposed separately to 2 females for 24 hr and reared at 27.5 $\pm$ 2°C, 80% RH, and 14 hr light until parasitoid emergence. Data relative to (1) time of development of the parasitoid from oviposition to adult emergence, (2) percentage of successful parasitism (= $\frac{\text{Parasitoid progeny emerged}}{\text{Parasitized host pupae recovered}}$ X 100), and (3) life span and morphological characteristics of the adult parasitoid progeny were collected.

Results

The index of host age preference by the female parasitoid, in the host choice experiment, is shown in Figure 9. The percentage of females observed on 5-day-old-host rings was higher throughout most of the period of observation. There was attraction also to 3-day-old-host larvae. A large proportion of females was observed on 6-day-old hosts initially,

Figure 9. Attraction (eye-fitted line) of T. daci females to 1-, 3-, 5- and 6-day-old A. suspensa host larva and host diet in a multiple choice test.


HOST AGE (DAYS)
1
3
5
6
HOST DIET
PERCENT PARASITOID FEMALES ATTRACTED
60
50
40
30
20
10
1
2
14
15
16
17
18
19
20
21
22
HOURS

but the proportion decreased during the last hours of observation. Almost no attraction was shown to 1-day-old larvae and host-contaminated diet.

Attraction to host ages was correlated with the mean number of parasitoids found per parasitized host and to the percentage of total parasitism in the dissected replicates (Figure 9). Mean values of 2, 2.6, 1.5 and 1.2 parasitoids per host were found in 6-, 5-, 3- and 1-day-old hosts, respectively.

Parasitoid development was affected by the host encapsulation reaction, and the proportion of eggs encapsulated in the dissected samples was correlated with host age and degree of superparasitism (Figure 10). Hence, due to these 2 effects the percentage of parasitoid adults that emerged was reduced in older hosts (Figure 11).

The percentages of successful parasitism and sex ratio of the surviving progeny of T. daci in the 1 through 6-day-old host exposed separately to parasitoid females are presented in Table 7. There was a decrease in progeny survival with increasing host age, but only the results from 1-day-old host were significantly higher ($P<0.05$) from all others. The differences in progeny survival between 1-, 2- and 3-day-old hosts were not significant ($P<0.05$). These results support the observations in the first experiment (simultaneous parasitization of several host ages) related to the unsuitability of older hosts. A considerable increase in the percentage of successful parasitism in the second experiment at all ages (Figure 11 and Table 7) might result from a different host parasitoid ratio, which is one of the reasons for reduction of superparasitism.

Figure 10. Percentage of superparasitism and encapsulation of T. daci by 1-, 3-, 5- and 6-day-old A. suspensa host larvae.

MEAN PERCENT SUPERPARASITISM
MEAN PERCENT ENCAPSULATION
10
20
30
40
50
60
70
80
90
1
3
5
6
HOST AGE (DAYS)
PERCENT ENCAPSULATION
PERCENT SUPERPARASITISM

Figure 11. Percentage parasitism and successful development of T. daci progeny in 1-, 3-, 5- and 6-day-old A. suspensa host larvae.

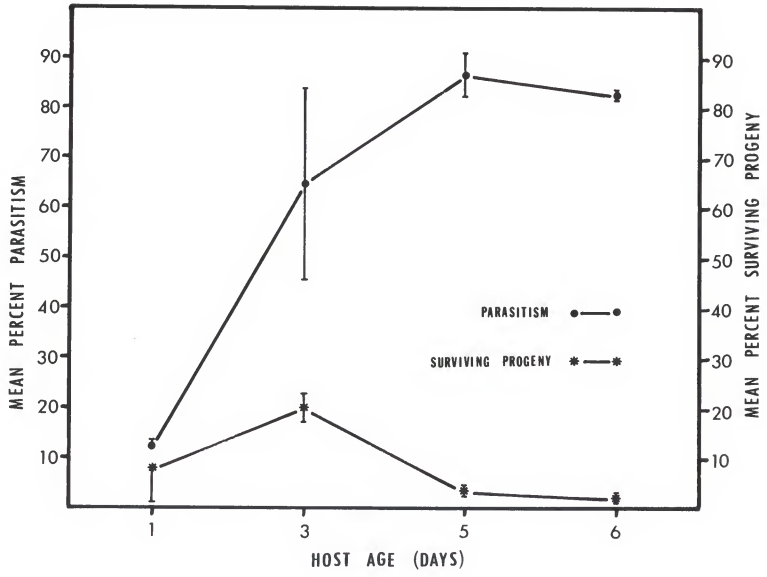

MEAN PERCENT PARASITISM
MEAN PERCENT SURVIVING PROGENY
HOST AGE (DAYS)
PARASITISM
SURVIVING PROGENY
90
80
70
60
50
40
30
20
10
1
3
5
6

Table 7. Percentage (X ± S.E.) T. daci progeny emerging from parasitized 1 - 6-day-old A. suspensa larvae, and host age effect on parasitoid sex ratio.

Host age (days)	% Parasitoids emerging	Parasitoid sex ratio ♀/♂
1	47.88 ± 5.88a[2/]	1.00/1.28
2	44.18 ± 5.39ab	1.06/1.00
3	47.11 ± 4.87ab	1.43/1.00
4	28.92 ± 1.20bc	1.00/1.61
5	17.90 ± 8.60c	1.50/1.00
6	19.58 ± 3.18c	1.70/1.00

1/ 100 larvae at each age were exposed to 2 females for 24 hr in each of 3 replicates.

2/ Mean values followed by the same letter were not significantly different ($P<0.05$) by Duncan's Multiple Range Test.

The sex ratio of the parasitoid progeny was imbalanced, and larger numbers of females emerged from older hosts (Table 7).

Size of the parasitoid progeny increased proportionally with size and age of the host but the length of the ovipositor was not affected (Table 8).

Other effects of the host age and size evaluated in the T. daci progeny such as the total number of mature eggs in nonovipositing females (Table 9) and longevity (Table 10) were not apparently different. Table 11 shows the average developmental time for male and female T. daci according to the age of the host at the time of parasitization. Results from the test of parasitization with different ages of A. suspensa host larvae are given in Table 12.

Discussion

The preference for older and larger larvae suggests a host-size cue for host finding by T. daci. Attraction to larger host was observed by Clausen et al. (1965) in laboratory reared T. daci released in the field. This is apparently a cue used by other cynipids (Havilan 1921) and was proven to be the most important for C. defonscolombie (Cynipidae) in the finding of syrphid host larvae (Rotheray 1979). The results presented by Bakker (1971) from the analysis of host selection by P. bochei are interesting. In this case the number of "hits" with the ovipositor was proportional to host surface area, but the actual number of eggs laid was related to the thickness of the cuticle. Histological

Table 8. Length in mm of T. daci male and female body and ovipositor, reared in 1 - 6-day-old A. suspensa larvae.

	Males		Females		Ovipositor	
Host Age	n	$\bar{x} \pm$ S.E.	n	$\bar{x} \pm$ S.E.	n	$\bar{x} \pm$ S.E.
1	19	2.59 ± 0.02	18	2.71 ± 0.27	18	2.36 ± 0.03
2	16	2.41 ± 0.37	16	2.85 ± 0.03	16	2.38 ± 0.04
3	15	2.60 ± 0.22	15	2.88 ± 0.02	15	2.36 ± 0.34
4	11	2.81 ± 0.18	9	2.92 ± 0.12	9	2.44 ± 0.09
5	15	2.87 ± 0.05	15	3.15 ± 0.05	15	2.50 ± 0.03
6	15	2.84 ± 0.06	15	3.15 ± 0.03	15	2.62 ± 0.04

Table 9. Number of eggs of T. daci females reared in 1 - 6-day-old A. suspensa larvae.

Host age (days)	n	$\bar{x} \pm$ S.E.
1	18	239.44 ± 7.34
2	28	270.75 ± 43.99
3	30	252.23 ± 6.63
4	8	243.37 ± 18.99
5	19	255.05 ± 8.33
6	23	273.69 ± 7.10

Table 10. Longevity in days of T. daci progeny reared in 1 - 6-day-old A. suspensa larvae.

Host age	Males			Females		
	n	$\bar{x} \pm$ S.E.	Range	n	$\bar{x} \pm$ S.E.	Range
1	11	8.27 ± 0.59	3-11	14	6.57 ± 0.85	2-11
2	31	14.58 ± 1.17	5-27	44	12.65 ± 1.13	5-27
3	16	10.93 ± 1.50	5-24	15	14.13 ± 1.61	5-24
4	25	13.40 ± 1.57	5-29	57	11.50 ± 0.94	5-29
5	18	11.44 ± 2.00	3-30	27	14.66 ± 5.19	3-30
6	23	11.78 ± 1.56	3-29	31	16.80 ± 5.14	3-29

Table 11. Rate of development in days of T. daci in 1 - 6-day-old A. suspensa host larvae.

Host age	Males			Females		
	n	$\bar{x} \pm$ S.E.	Range	n	$\bar{x} \pm$ S.E.	Range
1	64	27.36 ± 0.19	26-29	50	28.40 ± 0.09	28-31
2	65	26.88 ± 0.31	25-31	69	29.39 ± 0.49	26-34
3	53	26.13 ± 0.22	24-32	37	28.29 ± 0.08	26-32
4	50	25.12 ± 0.09	23-33	31	27.86 ± 0.70	25-30
5	18	28.64 ± 2.01	22-29	27	29.48 ± 1.32	24-34
6	21	26.43 ± 0.38	24-28	38	28.40 ± 0.15	26-32

Table 12. Results of parasitism by T. daci with different ages of A. suspensa host larvae.

Host age (days)	x Percentage ± S.E.[1/]			
	Host larval mortality	Host pupal mortality	Hosts yielding flies	Hosts yielding parasites
1	21.00 ± 2.94a[2/]	29.03 ± 6.19a	23.36 ± 2.18a	47.58 ± 5.88a
2	6.33 ± 0.72ab	41.52 ± 1.89a	14.25 ± 3.41a	44.18 ± 5.39ab
3	23.33 ± 4.38a	37.54 ± 5.58a	15.33 ± 0.94a	47.11 ± 4.87ab
4	6.33 ± 4.76b	30.02 ± 5.39a	40.92 ± 4.71b	28.92 ± 1.20abc
5	4.66 ± 3.81b	38.91 ± 7.52a	44.18 ± 4.43b	17.90 ± 8.60c
6	1.33 ± 0.54b	29.75 ± 1.53a	50.65 ± 3.27b	19.58 ± 3.18bc

1/ 100 larvae/age parasitized by 2 female parasitoids for 24 hr/3 replicates per host age.

2/ Numbers within the same vertical column followed by the same letter are not significantly different by Duncan's Multiple Range Test ($P<0.05$).

analysis revealed a thick cuticle in species and larval ages not accepted or accepted in lower degree as oviposition site by P. bochei.

The 6-day-old hosts were less well accepted by T. daci than were 5-day-old hosts, probably because of the normal changes in sclerotization of the cuticle. Both ages of hosts were equally attractive initially, probably because of their large size. Lower acceptance of smaller hosts reduced the percentage of parasitism in 1st and 2nd instar hosts (Figure 9 and 11). Lack of attraction to host contaminated diet may indicate no role in the choice of the host by host-emanated odors or by diet odors under laboratory conditions, but the situation in the field may be different. Clausen et al. (1965) observed that T. daci is attracted to rotten fruits. Other factors could be implicated in host age preference but they will require different experimental methods to be demonstrated. A major conclusion from the experiment is that the size of the host plays an important role.

Preference of the female parasitoid for older hosts did not correspond to the most suitable host. Similar attraction has been observed in other host:parasitoid systems. For example, C. victrix prefers older hosts but the encapsulation reaction was stronger in those hosts (Gutierrez 1970). Similar results were found in the choice of older C. capitata pupae by Miscidifurax raptor Girault and Sanders (Hymenoptera: Pteromalidae) which resulted in prolongation of developmental rate and skewed parasitoid sex ratio (Podoler and Mendel 1979). The effect of hosts on size of the parasitoids is an obvious influence and has been

the most frequently observed (Salt 1941). Lawrence et al. (1976) found increased size of B. longicaudatus progeny in relation to the host's larval age at the time of parasitization but this factor is apparently not important in mass rearing programs. The data for adult longevity are not very reliable for statistical comparison due to the effect of changes induced at room temperature where the individuals were maintained. The number of eggs present in the ovary was high. This is an indication of a high reproductive potential. The number of eggs and thus the potential progeny in Hymenoptera parasitoids are more related to the insect's taxon (Price 1972, 1973), and there appears to be no formal demonstration of the effect of the host size in this aspect (Salt 1941). Nevertheless, Salt (1935) demonstrated differences in the fecundity of T. evanescens in relation to the age of the host in which they were reared. The dissection of superparasitized host in the host choice experiment revealed prolongation of the 1st instar, and asynchrony in development of supernumerary parasites. Hosts with more than 3 parasitoids are more likely to succumb to the stress induced by the parasitization process, but in some cases 2 or 3 parasitoids reach prepupal stage within a normal appearing host. It would be expected to have prolonged parasitoid developmental time in older hosts if they were superparasitized, but the asynchrony in the rhythm of emergence might mask the results. Delayed development of parasitoids in older hosts has been demonstrated in parasitoids of dipterous host (Chabora and Pimentel 1966, Coats 1976, Lawrence et al. 1976, Podoler and Mendel 1979). The abnormal prolongation of immature stages may result from host-parasitoid hormonal interactions, also a parameter by which to judge the suitability of the host.

The sex ratio of the surviving T. daci progeny favored females when 3-, 5- and 6-day-old hosts were used for parasitization. No imbalance in favor of females was observed in the progeny of smaller hosts. This result requires further consideration due to its importance in mass production of parasitoids. Differences in sex ratio are related to the host's condition, sex and size (Clausen 1939), superparasitism (Salt 1934), environmental conditions, or the age of the parasitoid itself (Vinson 1980). These factors are difficult to discern in this experiment.

On the bases of the main parameters affected by the host age, that is, percentage of successful parasitism and sex ratio it is still difficult to identify an optimal host for mass production of T. daci.

A general analysis of host larval and pupal mortality (Table 12) indicates higher mortality in 1- and 3-day-old host that may result from the stress induced by the experimental procedures and effect of the parasitization. The total mortality (larval + pupal percentages) was lower in 4-, 5- and 6-day-old hosts. The mortality of older A. suspensa host is not important when the results are analyzed from the point of view of selection of a host age for mass production since they produce stronger reaction against the parasitoid and more flies emerged without being affected by the encapsulated parasitoids contained in the hemocoel.

A 2nd instar host larva is probably the best host since the production of parasitoids was not significantly different ($P<0.05$) from that observed in 1st instar. First instar host gave good development of the parasitoid but the 1st instar host larva is difficult to handle. Second instar larvae are easier to handle for experimental and rearing procedures.

T. daci adults emerging from 1-day-old larvae had a life span shorter than other adults, when all were maintained under similar conditions. The short life span of these adults may result from deficient nutrient reserves acquired during their development. This consideration and the possible costs of labor in the manipulation of a very small host larvae make it not acceptable for rearing procedures.

CHAPTER VI

INFLUENCE OF PARASITOID DENSITY AND HOST AGE UPON PARASITISM BY TRYBLIOGRAPHA DACI

In field situations and under laboratory conditions the efficiency of a parasitoid is represented by the number of eggs laid and distributed in suitable hosts. Solitary parasitoids prevent wastage of eggs by avoidance of superparasitism, which depends on the parasitoids' ability to discriminate between healthy and parasitized hosts and the capacity to restrain oviposition when suitable hosts are not available. The term host discrimination has been used in 2 ways: (1) the ability of a parasite to distinguish between parasitized and unparasitized hosts and lay eggs only in the former, and (2) the ability to distribute the eggs in a non-random manner among the hosts (Lenteren and Alphen 1978, Vinson 1976). Salt (1934) was the first to demonstrate host discrimination by comparison of the actual distribution of Collyria calcitrator (Grav) eggs in Cephus pygmaeus (L.) with a theoretical random distribution. Under laboratory conditions Salt (1937) studied the behavioral components of oviposition and discrimination by T. evanescens. Since then, many researchers have demonstrated or analyzed this ability in other parasitoids (Vinson 1976); however, the mechanism of host discrimination remains unknown (Fisher 1961, Vinson 1976).

Reduction in oviposition or migration from the potential host habitat results from specific behavioral factors induced by continuous

encounters with unsuitable or parasitized hosts or by interference with other females (Alphen 1980). Superparasitism, on the other hand, is mainly due to failure to restrain oviposition (Salt 1936), and its prevalence depends on the lack of availability of hosts (Fiske 1910).

The effects of superparasitism upon host and parasitoid progeny are diverse. One of the most common outcomes is the death of hosts and supernumerary parasitoids. The supernumerary parasitoids eliminate each other by physical attack, by physiological suppression or by competition for food leading to the survival of one parasitoid (Salt 1961, Fisher 1971). The surviving progeny, if any, are severely affected and the sex ratio is unbalanced, usually in favor of males (Salt 1936). Other factors, especially host defenses and environmental conditions, become the ultimate determinants of parasitoid survival to the adult stage.

In early experiments it was observed that from every host pupa only one T. daci adult emerged. Nevertheless, dissection of superparasitized hosts often showed more than one parasitoid in development. Successful parasitism and analysis of factors causing parasitoid and host mortality are important in the determination of the host age and host-parasitoid ratio to optimize the parasitization effect. This experiment was undertaken to evaluate the influence of different parasitoid densities on egg distribution by 5 - 6-day-old T. daci females with a constant number of 2nd and 3rd instar A. suspensa larvae, and the effect of encapsulation as a function of host age and superparasitism upon the successful development of the parasitoid progeny.

Materials and Methods

A. suspensa larvae were reared following the general procedures explained before, at 27.5 ± 2°C, 50-70% RH, and 14 L:10 D. The T. daci adults were taken from the general stock colony (reared in 3rd instar hosts) after 24 - 48 hr of oviposition experience.

Second instar host larvae (3 days old) and 3rd instar host larvae (5 days old) were exposed (400 at each age) separately to 4, 8, and 32 5 - 6 day old parasitoid females (low, medium and high parasitoid densities). These proportions gave a ratio of 100, 50 and 12.5 hosts per female parasitoid. The experiment was replicated 4 times. The hosts were presented in embroidery hoop sting units to females confined in parasitization cages (36 x 36 x 18 cm) for 24 hr. After pupation, the parasitized hosts were divided into 4 groups containing approximately the same number each. The first group was dissected 5 - 6 days after parasitization and the second 10 - 12 days after the same date. They were dissected in vivo to observe the stage and condition of the parasitoid. The number of parasitoids per host in the first group determined the egg distribution for each parasitoid density and host age. The observed distribution was compared with a theoretical random (Poisson) distribution by chi-square analysis (Wadley 1967) in order to detect host discrimination by the female parasitoids. The total number of immature parasitoids indicated the oviposition rate at each parasitoid density. The number of nonencapsulated and encapsulated parasitoids in solitary and superparasitized hosts in the second group of hosts dissected at 10 - 12 days was noted.

The remaining groups (reared control grous) were allowed to continue their development until flies or parasitoids emerged. The number of parasitoids reaching the adult stage was used to evaluate the effect of host density and host age on successful parasitism. The effect of rearing conditions and manipulation upon host mortality was evaluated in control host larvae not exposed to parasitoids.

Results

The distribution of parasitoids in 2nd and 3rd instar hosts is presented in Tables 13 and 14. Significant chi-square values indicate that T. daci distributed its eggs in a non-random manner. The chi-square values for 2nd instar hosts (Table 13) were highly significant ($P<0.01$) at medium density, and distribution tended to be random as parasitoid density increased or decreased. At lower density a female was provided with more hosts and tended to leave 1 egg per host, but more hosts were unparasitized. Concomitant with that, superparasitism decreased from 21.3% at medium density to 13.3% at lower density. Lack of significant chi-square values at higher parasitoid density indicates that females were not provided with sufficient numbers of hosts, but even under those circumstances discrimination occurred in some replicates. Superparasitism (68.8%) was 3 times higher than that observed at medium density.

The distribution of parasitoids in 3rd instar hosts (Table 14) was not significantly different from a Poisson distribution in all replicates at medium density, but still the number of hosts with 1 parasitoid was larger than that observed in the 2 other densities. The non-significant value at lower density did not necessarily mean inability of the female

Table 13. Distribution of eggs by 5 - 6-day-old T. daci females in 2nd instar A. suspensa larvae during 24 hr at different parasitoid densities.

Replicate	Number of Hosts with n parasitoids							Parasitoid eggs		S^2	Calculated chi-square
	0	1	2	3	4	>5	Total	Total	$\bar{x}$/host		
4 Parasitoids:4 hosts											
1	25	50	13	4	0	1	93	93	1.00	0.76	11.2*[1/]
2	24	64	3	0	0	0	91	70	0.77	0.24	45.7**
3	48	37	7	0	0	0	92	51	0.55	0.40	2.6 ns
4	25	58	2	2	0	0	87	68	0.78	0.35	15.2**
8 Parasitoids:400 hosts											
1	10	45	18	14	6	3	96	165	1.71	1.17	18.87**
2	14	50	10	4	1	1	91	91	1.13	0.80	24.82**
3	36	51	1	1	0	0	89	56	0.57	0.32	25.72**
4	10	67	5	2	0	0	84	83	0.98	0.27	65.31
32 Parasitoids:400 hosts											
1	1	12	24	14	11	25	87	309	3.55	3.55	20.0**
2	11	15	31	12	10	12	91	219	2.40	2.79	11.1 ns
3	12	43	18	8	2	2	85	121	1.42	1.15	12.45*
4	9	32	26	12	7	1	87	153	1.75	1.30	4.9 ns

1/ Observed distribution ns not significantly different (P>0.05), * significantly different (P<0.05) and ** significantly different (P<0.01) from a Poisson distribution.

Table 14. Distribution of eggs by 5 - 6-day-old females in 3rd instar A. suspensa larvae during 24 hr at 3 parasitoid densities.

Replicate	Number of hosts with n parasitoid eggs							Parasitoid eggs		s^2	Calculated chi-square
	0	1	2	3	4	>5	Total	Total	$\bar{x}$/host		
4 Parasitoids:4 hosts											
1	72	20	5	2	0	0	99	36	0.36	0.45	5.0**[1/]
2	35	23	9	0	0	0	67	41	0.61	0.51	1.0 ns
3	45	36	6	0	0	0	87	48	0.55	0.38	3.3 ns
4	46	39	7	2	0	0	94	59	0.62	0.51	2.9 ns
8 Parasitoids:400 hosts											
1	31	50	18	0	1	0	100	90	0.90	0.57	7.3*
2	15	26	24	10	2	0	77	112	1.45	1.06	3.8 ns
3	18	59	19	4	0	0	100	109	1.19	0.52	22.1**
4	20	44	20	4	2	0	90	104	1.15	0.80	7.86*
32 Parasitoids:400 hosts											
1	23	39	26	7	1	1	100	133	1.33	1.19	2.16 ns
2	14	6	13	15	7	4	59	132	2.23	3.59	15.5 ns
3	10	32	34	15	3	0	94	157	1.67	0.95	8.49*
4	11	29	18	22	8	9	97	220	2.26	3.07	19.0*

[1/] Observed distribution is not significantly different ($P>0.05$), * significantly different ($P<0.05$) and ** significantly different ($P>0.01$) from a Poisson distribution.

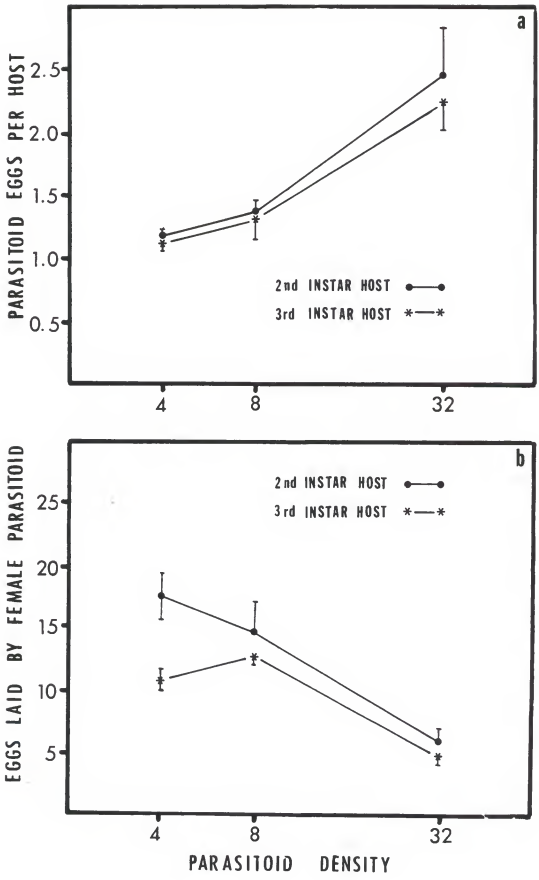
to recognize already parasitized hosts. The data indicated a tendency for females to leave 1 egg per host, but many of the hosts were not parasitized. The degree of superparasitism increased in relation to that observed in 2nd instar hosts at each corresponding density. The average percentage from 4 replicates was 21.7, 37.7 and 66.04 for low, medium and high densities.

The differences between the number of 2nd and 3rd instar hosts that did not receive parasitoid eggs indicated lower acceptability of the older host (3rd instar, 5 days old). The percentages of nonparasitized 2nd instar hosts were 33.5, 20.0 and 9.4 at low, medium and high densities, and 56.4, 22.6 and 17.8 for 3rd instar hosts, respectively. Lower acceptance of 3rd instar host larvae might be explained on the basis of physiological changes in the host larvae induced by variable conditions in the rearing room of the fruit fly colony, so that the larvae were not uniform in all replicates. Another reason for the differences might be the normal maturation of these larvae during the time of exposure to the parasitoid.

The mean number of T. daci eggs per host increased as a function of parasitoid density (Figure 12a), an indication of a tendency towards superparasitism. This resulted from continuous encounters over a long time of the females with parasitized hosts. Probably the females detected the parasitized condition but laid the egg in spite of the fact because of an absence of suitable hosts. Nevertheless, each female laid fewer eggs when hosts were not available (Figure 12b).

The mean number of eggs laid by females (Figure 12b) showed a clear decline when parasitoid density increased. The decrease in ovi-

Figure 12. (a) Mean number of T. daci eggs per 2nd and 3rd instar A. suspensa hosts.
(b) Mean number of eggs laid by female parasitoid per day in 2nd and 3rd instar hosts at 3 parasitoid densities.


a
PARASITOID EGGS PER HOST
2.5
2.0
1.5
1.0
0.5
2nd INSTAR HOST
3rd INSTAR HOST
4
8
32
b
EGGS LAID BY FEMALE PARASITOID
25
20
15
10
5
2nd INSTAR HOST
3rd INSTAR HOST
4
8
32
PARASITOID DENSITY

position might result from rejection of already parasitized hosts, or from inhibitory influence of other parasitoids. The reduction of oviposition might prove that females interfere with each other, so that only stronger competitors have access to the host.

The mean percentage of parasitism (= $\frac{\text{Actual parasitized hosts}}{\text{Total hosts dissected}}$ X 100) increased with parasitoid density, and it was significantly lower only at the lower level for both host ages (Figure 13a and 13b). The percentage, however, of successful parasitism (= $\frac{\text{Parasitoid progeny emerged}}{\text{Pupae recovered}}$ X 100) in the reared groups (= nondissected controls) was not statistically different at any level within the same host age. However, the means were significantly higher for 2nd instar hosts compared to the means for 3rd instar hosts.

A clear effect of host age and parasitoid egg distribution was observed in the actual parasitoid progeny recovered from each female parasitoid. The yield (= $\frac{\text{Parasitoids emerged from reared pupae}}{\text{Parasitoid eggs in dissected samples}}$ X 100) decreased with increasing density, and consequently with the host available per female. The tendency was the same in both ages (Figure 14a and 14b). The means in 3rd instar hosts were all significantly lower than those at the same density level in 2nd instar hosts. The mean yield for 2nd instar hosts at low and medium density were significantly larger than that observed at higher density.

Encapsulation (percentage of parasitoids encapsulated) was significantly lower against solitary parasitoids in 2nd instar hosts at low and medium density (Table 15), but no significant differences ($P<0.05$)

Table 15. Influence of A. suspensa larval age and 5 - 6-day-old T. daci density upon percentage of encapsulation.

Host Instar	Parasitoid Density	Host:parasitoid Ratio	$\bar{x}$% (± S.D.) Parasitoid encapsulated[1/]	
			Host with 1 parasitoid	Host with >1 parasitoid
2nd	4	100:1	19.5 ± 3.65a[2/]	78.2 ± 10.09c
	8	50:1	25.5 ± 10.60a	79.1 ± 13.98c
	32	12.5:1	50.4 ± 14.52b	85.81 ± 4.98c
3rd	4	100:1	93.92 ± 3.85c	96.52 ± 3.61c
	8	50:1	92.06 ± 6.35c	94.61 ± 6.64c
	32	12.5:1	96.43 ± 2.73c	96.96 ± 1.27c

1/ Average from 4 replicates per density in host pupae dissected 5 - 6 days after parasitization.

2/ Means followed by the same letter are not significantly different ($P<0.05$) by Duncan's Multiple Range Test.

Figure 13. Percentage parasitism by T. daci and percentage progeny survival in A. suspensa larvae at 3 parasitoid densities in 2nd instar hosts (a) and in 3rd instar hosts (b).

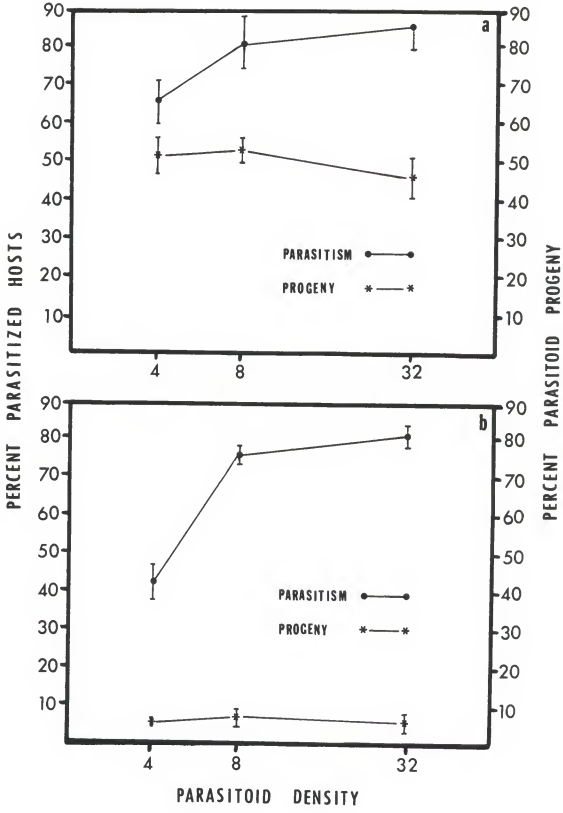
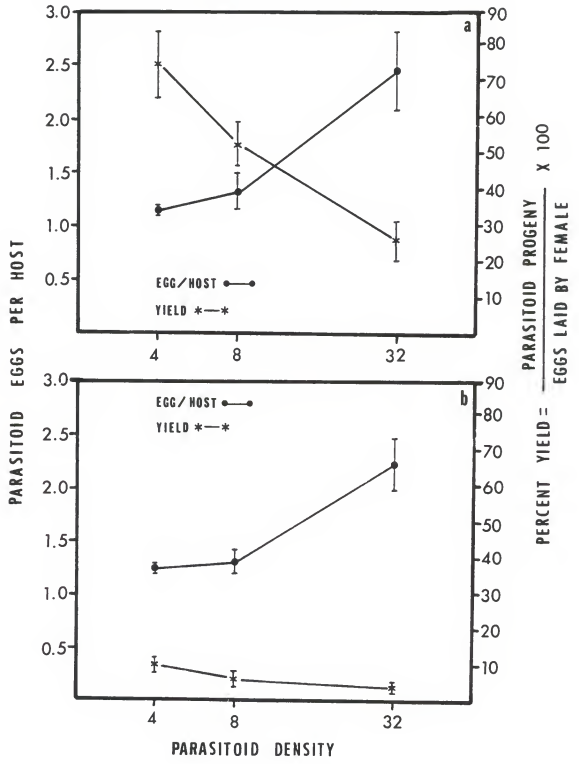

a
b
PARASITISM
PROGENY
PERCENT PARASITIZED HOSTS
PERCENT PARASITOID PROGENY
PARASITOID DENSITY
4
8
32
10
20
30
40
50
60
70
80
90

Figure 14. Distribution of T. daci eggs and percentage of parasitoid yield in A. suspensa larvae at 3 parasitoid densities (a) in 2nd instar hosts and (b) in 3rd instar hosts.


a
b
EGG/HOST
YIELD
3.0
2.5
2.0
1.5
1.0
0.5
90
80
70
60
50
40
30
20
10
4
8
32
PARASITOID EGGS PER HOST
PERCENT YIELD = PARASITOID PROGENY / EGGS LAID BY FEMALE X 100
PARASITOID DENSITY

in encapsulation were found in 2nd instar hosts at higher parasitoid density and with the means of encapsulation by 3rd instar host larvae. An increased tendency for hosts to encapsulate solitary parasitoids within the same host age, at increased parasitoid adult density, might indicate activation of host defenses due to stress caused by oviposition attempts by larger numbers of females. In superparasitized hosts the total reaction was invariable and equally high for both ages and densities (Table 15).

The total proportion of nonencapsulated parasitoid eggs and the distribution of encapsulation in superparasitizeds hosts from 4 replicates and host ages (Table 16, 17, 18 and 19), indicate an increased tendency of encapsulation and superparasitism. The differences within the same host age at different times of dissection (see Table 16 and 17; and 18 vs 19) might result from the evasion of host encapsulation by active movements of the emerging parasitoid, reactions that were frequently observed. The ability to overcome encapsulation was lower in 3rd instar hosts; however, the data were not completely valid for statistical comparisons because of the uncertainty that hosts containing one parasitoid in the second week after parasitization might have had more than one parasitoid initially.

The state and condition (dead or alive) of supernumerary T. daci nonencapsulated in superparasitized 2nd instar hosts at different times of dissection are presented in Table 20. In hosts dissected 5 - 6 days after parasitization 2 or 3 1st instar larvae were found, and in some cases one was dead, but there was no evidence of physical attack. However, in dissections made during the life cycle study, I observed 3

TABLE 16. Total percentage, from 4 replicates of non-encapsulated (0) and encapsulated (1-10) T. daci[1] by 2nd instar A. suspensa larvae at 3 parasitoid densities.

Parasitoids per host	total parasitoids	Encapsulated parasitoids per host									
		0	1	2	3	4	5	6	7	8	10
						4 Parasitoids: 400 Hosts					
1	209	80.4	19.6								
2	50	24.0	20.0	56.0							
3	18	27.8	5.5	3.3							
5	5	0.0			100.0						
						8 Parasitoids: 400 Hosts					
1	213	73.7	26.9								
2	68	20.6	20.6	50.0							
3	63	11.1		76.2							
4	28	10.7			32.14	57.1					
5	10	0.0					100.0				
6	6	0.0						100.0			
7	7	28.57							71.4		
						32 Parasitoids: 400 Hosts					
1	102	52.9	46.2								
2	198	20.2	17.2	67.6							
3	138	14.2	2.9	6.5	76.1						
4	120	13.3		6.6	12.5	66.6					
5	100	4.0			3.0	8.0	85.0				
6	54	16.6			5.5	14.8	18.51	44.4			
7	28	0.0							100.0		
8	32	0.0								100.0	
10	30	0.0									100.0

[1] Dissected at 5-6 days of age.

TABLE 17. Total percentage from 4 replicates of non-encapsulated (0) and encapsulated (1-10) T. daci[1/] by 2nd instar A. suspensa larvae at 3 parasitoid densities.

Parasitoids per host	total parasitoids	Encapsulated Parasitoids per host						
		0	1	2	3	4	5	6
				4 Parasitoids: 400 Hosts				
1	268	87.7	12.3					
2	40	35.0	15.0	50.0				
3	3	33.0			66.6			
4	4	0.0				100.0		
				8 Parasitoids: 400 Hosts				
1	252	89.9	10.7					
2	64	50.0	37.5	56.2				
3	30	10.0	0.30	6.6	80.0			
4	8	37.5	12.50			50.0		
5	5	0.0					100.0	
				32 Parasitoids: 400 Hosts				
1	185	89.2	10.8					
2	110	24.5	15.4	60.0				
3	135	13.3	4.4	8.8	73.3			
4	104	16.3	0.0	1.9	11.5	69.2		
5	60	18.3			1.0	13.3	58.3	
6	18	0.0						100.0

[1/] Dissected at 10-12 days of age.

TABLE 18. Total percentage from 4 replicates of non-encapsulated (0) and encapsulated (1-10) T. daci[1] by 3rd Instar A. suspensa larvae at 3 parasitoid densities.

Parasitoids per host	Total parasitoids	Encapsulated parasitoids per hosts									
		0	1	2	3	4	5	6	7	8	10
					4 Parasitoids: 400 Hosts						
1	118	5.1	94.9								
2	54	3.7	3.7	92.6							
3	12	0.0			100.0						
					8 Parasitoids: 400 Hosts						
1	178	7.3	92.13								
2	164	419	1.21	93.9							
3	54	0.0			100.0						
4	16	0.0				100.0					
					32 Parasitoids: 400 Hosts						
1	106	4.7	95.3								
2	182	3.3	3.0	94.5							
3	177	0.6		1.1	98.3						
4	88	5.7		2.3	10.2	81.8					
5	20	0.0					100.0				
6	30	0.0						100.0			
7	21	0.0							100.0		
8	8	0.0								100.0	
10	10	0.0									100.0

[1] Dissected at 5-6 days of age.

TABLE 19. Total Percentage from 4 replicates of non-encapsulated (0) and encapsulated (1-10) *T. daci*[1] by 3rd Instar *A. suspensa* Larvae at 3 Parasitoid Densities.

Parasitoids per hosts	Total parasitoids	Encapsulated parasitoids per hosts									
		0	1	2	3	4	5	6	7	8	10
						4 Parasitoids: 400 Hosts					
1	133	24.8	75.2								
2	64	4.7	4.7	90.6							
3	24	8.3		16.6	75.0						
4	4	0.0				100.0					
						8 Parasitoids: 400 Hosts					
1	195	19.5	80.5								
2	154	9.1	5.2	85.7							
3	51	5.8	3.9	3.9	88.2						
4	28	3.6			10.7	85.7					
						32 Parasitoids: 400 Hosts					
1	121	30.6	69.4								
2	196	9.2	7.1	83.6							
3	120	7.5	1.6	3.3	87.5						
4	116	11.2		6.9	2.6	79.3					
6	30	0.0						100.0			
7	14	0.0							100.0		
8	16	0.0								100.0	
10	10	0.0									100.0

[1] Dissected at 10-12 days of age.

cases in which the primary larva had wounds, and in a few cases 2 encapsulated parasites were enclosed in the same group of melanotic cells. In hosts dissected 10 - 12 days after parasitization up to 5 larvae were found within the same host (Table 20), and they were delayed in development.

Two hosts with 2 prepupae and 2 with 2 pharate adults were found in which the female was the smaller but apparently the victor because the female was completely developed, whereas the male was poorly developed.

Discussion

The results regarding host discrimination, superparasitism and total oviposition are consistent with findings in similar studies in other parasitoid systems (Salt 1936, Burnet 1958, Bakker at al. 1967, Lawrence et al. 1978). Salt (1936) studied the effects of T. evanescens density on constant number of Sitotroga sp. eggs, and concluded a perfect ability of the parasitoid to discriminate but the restraining capacity was limited and a breakdown occurred after prolonged contact with parasitized hosts. Bakker et al. (1967) observed decreased oviposition of female P. bochei with similar parasitoid ratios to those used in the present experiment, and found the same tendency in overall oviposition. They demonstrated negligible interference between females during oviposition, and decrease in fecundity was ascribed to rejection of already parasitized hosts. In B. longicaudatus reduction in oviposition resulted from aggressive behavior between conspecifics and larger females disrupted weaker competitors (Lawrence 1981b). I never

Table 20. Instar (I, II, III) and condition (A, alive; D, dead) of non-encapsulated T. daci in superparasitized A. suspensa 2nd instar host larvae.

Parasite age at dissection (days)	Superparasitized hosts dissected		Non-encapsulated parasitoids per superparasitized hosts		
	Parasitoids per hosts	Total hosts	n	(Instar and condition)[1/]	Frequency
5 - 6	2	158	1	I (A) + 1 I (D)	3
			2	I (A)	
	3	73	2	I (A)	2
			2	I (D)	1
			3	I (A)	1
			1	I (A) + 1 I (D)	2
			2	I (A) + 1 I (D)	1
			1	I (A) + 2 I (D)	2
	4	37	2	I (A)	3
			2	I (D)	1
			2	I (A) + 1 I (D)	1
	5	20	3	I (A)	2
	6	10	2	I (A) + 1 I (D)	1
10 - 12	2	107	1	I (A) + 1 II (A)	1
			1	II (A) + 1 III (A)	1
			2	II (A)	2
			2	III (A)	3
	3	56	1	I (A) + 1 II (A)	1
			2	II (A)	1
			2	III (A)	4
	4	29	2	II (A)	1
			3	II (A)	1
			4	II (A)	2
			1	II (A) + 2 III (A)	1
	5	13	1	I (D) + 1 II (A)	
				+ 3 III (A)	1
			1	II (A) + 1 III (A)	1

observed aggressive behavior in T. daci but there is no data on other kinds of interference. The variance of egg distribution (Table 13 and 14) was larger at the highest parasitoid density. Similar results were also observed by Bakker et al. (1967) in P. bochei.

An inverse relationship between progeny production and parasitoid density was observed in B. longicaudatus (Ashley and Chambers 1979) and in T. evanescens (Salt 1936), and the effect resulted from the stress caused by ovipositing females that laid more eggs or probed in already parasitized hosts, causing the death of the host. In T. daci lower progeny production at higher parasitoid density resulted from the effect of reaction of the host hemocytes. Encapsulation was higher in older and superparasitized hosts. This supports previous observations. Increased encapsulation as a function of age has been observed with lepidoptera hosts (Putler 1961, Lewis and Vinson 1971, Lynn and Vinson 1967). Walker (1959) found that encapsulation of P. bochei was less effective in 1st and 2nd instar Drosophila sp larvae. Lower encapsulation in younger hosts has been associated with inadequate number of hemocytes (Putler 1961, Lewis and Vinson 1971) or with lack of proper hemocytes for encapsulation (Walker 1959).

The evasion of host defenses by parasitoids has been explained in several ways. Walker (1959) suggested that P. bochei actively suppressed the encapsulation reaction of Drosophila larvae. Other parasitologists (Streams and Greenberg 1969, Nappi and Streams 1969, Nappi 1973, 1975b) supported Walker's (1959) hypothesis. The inhibitory factor, it has been suggested, is injected by the female parasitoid and works via the neuroendocrine system. That factor prevents transformation of hemocytes

(plasmatocytes into lamellocytes) necessary for encapsulation. According to general opinion, oviposition in younger hosts could provide the necessary time to inhibit the host defenses. On the other hand, if parasitization occurs in later host stages the mature hemocytes will be already present and the inhibitory substance is no longer active. Salt (1973) suggested that parasitoids avoid defensive reaction of their hosts by means of a protective surface. Nemeritis (=Venturia) canescens eggs, for example, were protected by a particulate fluid produced in the calyx of the female parasitoid. Vinson (1972b) and Vinson and Scott (1975) found Deoxyribonucleic acid (DNA) - virus-like particles produced in the calyx of C. nigriceps. Similar secretions were found in several species of braconids (Stoltz et al. 1976, Stoltz and Vinson 1976). More recent studies (Edson et al. 1980) proved the active inhibition of H. virescens defensive reactions by C. sonorensis (Icheumonidae). The inhibitory factor is a DNA-baculo virus-like that replicates in the calyx cells and is injected by the female parasitoid in the host.

Superparasitism also influenced the proportion of encapsulated T. daci eggs. Salt (1963) and Kitano (1969), suggested that only dead or dying supernumerary parasitoids are encapsulated. In general, heavily parasitized hosts are not able to encapsulate the supernumeraries because there is inhibition or debilitation of host defenses, or limited availability of hemocytes (Salt 1963, Putler and Van dem Bosch 1959). Steams (1971) studied the encapsulation of P. bochei by D. melanogaster in solitary and superparasitized hosts and observed lower encapsulation as superparasitism increased. The same host was able to encapsulate all

supernumeraries P. mellipes eggs, and up to 7 capsules were found per host (Streams and Greenberg 1969). The encapsulation of supernumerary T. daci eggs is similar to that observed in P. mellipes. I found up to 10 capsules per A. suspensa pupa, independent of the host age at parasitization. Nevertheless, some supernumeraries escape from encapsulation. These results do not suggest debilitation of the host defenses.

Physical attack is usually observed in the 1st instar of Hymenoptera parasitoids as a way of elimination of supernumerary parasitoids (Salt 1961), but in order for it to occur the larvae should emerge at the same time and be physically in contact. It is possible this mechanism takes place in T. daci, but the encounter did not occur because the 1st instar parasitoid could emerge over a period of 24 hr and eggs were laid in different parts of the host's body. Jenni (1951) suggested that physical attack and physiological suppression by a metabolic enzyme that kills the contender or blocks its digestion were the main ways of elimination in P. bochei. Studies by Eijsacker and Bakker (1971b) with the same species demonstrated that physical attack was important but other means to eliminate advanced stages could exist. The coexistence of supernumeraries in advanced stages of development does not allow one to rule out physiological suppression as a way of elimination in T. daci. This is a field that has not been studied extensively in cynipids.

Superparasitism did not affect the sex ratio of T. daci progeny. Females with deformed antennae and small bodies were only observed at higher parasitoid densities at which the incidence of superparasitsm was also higher. According to Salt (1936) superparasitism may cause imbalance of sex ratios, usually in favor of males. This effect seems not

to be applicable to T. daci, since the proportion of males to females was maintained in equilibrium. The observed female:male ratio in the surviving progeny was equal to 1.0:1.3, 1.4:1.0 and 1.2:1.0 in 2nd instar hosts and 1.0:1.3, 1.2:1.0 and 1.0:1.0 in 3rd instar hosts, respectively, in order of increasing parasitoid density.

Parasitoid density also influenced host mortality. As expected, high density caused more stress on the host. The percentage of mortality (host larvae + pupae) for 2nd instar hosts was 26.3, 34.7 and 43.1% for low, medium and high densities. The proportion, in the same order for 3rd instar hosts was equal to 44.0, 39.2 and 51%. These values include mortality in the absolute control calculated to be 11%. The differences in mortality between hosts ages at the time of parasitization suggest that larger hosts were objects of more attacks and probing than younger hosts, although older hosts were less acceptable for oviposition, (Figure 12b), since overall oviposition per female was inferior in older hosts.

The results from this experiment supported the previous conclusion that younger hosts are better for mass production of T. daci. Another clear conclusion is the advantage of using few parasitoids per rearing cages to avoid superparasitism and/or host and parasitoid mortality as a result of stress caused by ovipositing females. A higher yield of parasitoids can be produced at lower parasitoid density, but the loss of nonparasitized hosts could be an economic disadvantage in mass rearing. Additional research is justified to determine the more economic host:parasitoid ratio.

CHAPTER VII

INFLUENCE OF HOST DENSITY UPON PARASITISM BY TRYBLIOGRAPHA DACI

Host density refers to the number of hosts present in a defined space per female parasitoid. The conventional experimental procedure of leaving the parasitoid with an established number of hosts during a certain time allows an evaluation of egg distribution and progeny per female and the parasitoid response to variations of the number of available hosts (Flanders 1935, Burnet 1958, Podoler and Mendel 1979). The results under laboratory conditions may differ from those observed under field situations but still provide the information necessary to standardize a host parasitoid ratio for mass rearing procedures, by means of which a maximum number of parasitoid progeny can be recovered and the lowest number of hosts left unparasitized.

In preliminary experiments it was found that T. daci females 5 - 6 days old distributed their progeny in a discriminatory way. The number of progeny recovered increased when the number of hosts available per female was larger than 50. A high parasitoid density, however, for example, impaired parasitoid efficiency so that it did not reach the maximum reproductive potential. Stress by a large number of ovipositing females on a constant number of hosts caused a reduction in the surviving progeny per female. The maximum percentage of surviving progeny was obtained at a host:parasitoid ratio of 100:1, but many hosts were left unparasitized.

The following experiment was designed to evaluate the effect of increasing the number of A. suspensa 2nd instar larvae (= host density levels) on egg distribution and unsuccessful development of T. daci.

Materials and Methods

Adult parasitoids were collected from the stock colony during an emergence period of 12 hr, and exposed to 2nd instar host larvae for 6 hr in the fourth day. Following 6 hr oviposition experience, the adult parasitoids, now 5 days old, were used to evaluate the effect of host density on parasitism. Second instar host larvae were exposed in agar-based parasitization units (14.5 cm diameter x 1.5 cm h) containing each 100, 200, 300 and 400 host larvae. Four female parasitoids were allowed to oviposit for 24 hr in each unit. These arrangements provided a host:parasitoid ratio of 25:1, 50:1, 75:1 and 100:1. After parasitization the host larvae were manipulated as explained in the general rearing methods. A sample of parasitized hosts equivalent to 25% of the initial number exposed at each host density was preserved in 70% ethanol for later dissection. The rest were reared at 27.5 $\pm$ 2°C, 70-80% RH and 14 hr photophase until parasitoid emergence. A control group of hosts not exposed to the parasitoids, per treatment, was reared under the same conditions.

Results

The observed distribution of eggs by T. daci females was significantly different from the calculated random (= Poisson) distribution at all host levels above 200 (Table 21). These significant values indicate

Table 21. Distribution of eggs by 4 T. daci females[1/] in 2nd instar A. suspensa larvae at different host densities.

Replicate	No. of hosts with n parasitoid eggs						No. of parasitoid eggs		s^2	Calculated Chi-square
	0	1	2	3	>4	Total	Total	$\bar{x}$/host		
						100 hosts:4 parasitoids				
1	1	4	7	8	5	25	63	2.52	1.42	2.49 ns[2]
2	1	11	7	2	4	25	49	1.96	1.79	5.61 ns
3	3	13	5	4	0	25	35	1.49	0.83	4.51 ns
						200 hosts:4 parasitoids				
1	13	34	2	2	0	50	41	0.82	0.35	22.23**
2	14	30	6	0	0	50	42	0.84	0.38	10.77**
3	10	36	4	0	0	50	44	0.88	0.27	19.28**
						300 hosts:4 parasitoids				
1	22	43	7	2	1	75	67	0.89	0.61	14.35**
2	31	44	0	0	0	75	44	0.58	0.24	18.78**
3	36	37	2	0	0	75	41	0.54	0.30	11.78**
						400 hosts:4 parasitoids				
1	36	62	2	0	0	100	66	0.66	0.26	34.87**
2	29	66	5	0	0	100	76	0.76	0.28	36.52**
3	31	65	4	0	0	100	73	0.73	0.28	34.97**

1/ 6 hours of oviposition experience.

2/ Observed distribution ns not significantly different ($P>0.05$), * significantly different ($P<0.05$) and ** significantly different ($P<0.01$) from a Poisson distribution.

that females discriminate between parasitized and non-parasitized hosts (Wadley 1967). The number of hosts available per female was reduced at low host density, and masked the parasitoid discriminatory ability.

The total number of eggs laid by female parasitoids was significantly higher only at the highest density when hosts available per female were equal to 100:1 (Table 22). No statistical differences were found between the other means. The mean number of eggs per parasitized host decreased with increasing host density and the average was significantly higher only at the lowest density. A similar tendency was observed in the percentage of superparasitized host as shown in Table 22. These results agree in general with the tendency observed in the previous experiment. The increased number of hosts available stimulated the females to lay more eggs and distribute them in a discriminatory way.

The estimated number of hosts attacked (solitary plus superparasitized) per female per day increased with increasing host:parasitoid ratio as indicated in Figure 15a, but the percentage of parasitized host per female ($=\frac{\text{Number of parasitized hosts}}{\text{Total number of dissected hosts}} \times 100$) at each host parasitoid ratio decreased in relation to the number of hosts available per female (Figure 15b).

Holling (1959a) described the feeding rate (prey death rate) by an individual predator (parasitoid) in response to prey or host density as the functional response. The experimental procedure to analyze the functional response should include the observation of the activity of the parasitoid in response to prey density and distribution, in order to determine the number of hosts attacked per unit of time. This procedure

Table 22. Effect of host density[1] on total oviposition, number of eggs per host and percent superparasitism by female T. daci that were 5 days old with 6 hr of oviposition experience.

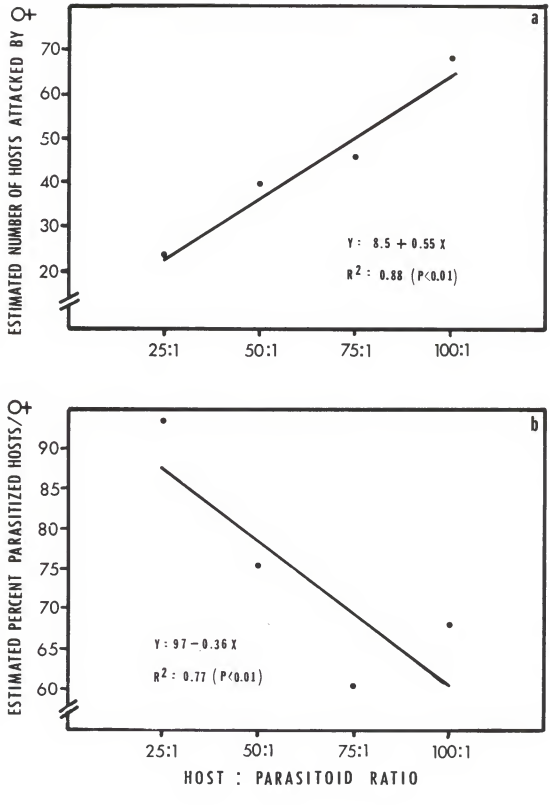
Host density	Eggs deposited /♀/day $\bar{x} \pm$ S.E.	Eggs/host/day $\bar{x} \pm$ S.E.	Percent superparasitism $\bar{x} \pm$ S.E.
100 (25:1)[2]	49.00 ± 6.6a[3]	2.1 ± a	59.4 ± 10.20a
200 (50:1)	42.30 ± 0.7a	1.1 ± b	11.5 ± 2.11b
300 (75:1)	50.60 ± 6.7a	1.1 ± b	8.0 ± 4.60b
400 (100:1)	71.66 ± 2.4b	1.0 ± b	5.3 ± 0.90b

[1] Average of 3 replicates per host density.

[2] Host:parasitoid ratio.

[3] Means followed by the same number in the same column were not significantly different (P<0.05) by Duncan's Multiple Range Test.

Figure 15. (a) Estimated number of eggs laid by a 5-day old female T. daci.
(b) Percentage parasitism in 2nd instar A. suspensa larvae at 4 host:parasitoid ratios.


a
ESTIMATED NUMBER OF HOSTS ATTACKED BY ♀
70
60
50
40
30
20
Y: 8.5 + 0.55 X
R2: 0.88 (P<0.01)
25:1
50:1
75:1
100:1
b
ESTIMATED PERCENT PARASITIZED HOSTS/♀
90
85
80
75
70
65
60
Y: 97 − 0.36 X
R2: 0.77 (P<0.01)
25:1
50:1
75:1
100:1
HOST : PARASITOID RATIO

allows one to calculate the rate of searching and handling time. The experimental procedure used with T. daci in this experiment did not consider the evaluation of these parameters. The results presented in Figure 15a and 15b may represent a part of the functional response of T. daci at high host densities. The lowest density of 25 hosts per female parasitoid might be too high to detect the increasing attack rate of the parasitoid at lower densities and masked the actual functional response. The results were biased by the interference from other ovipositing females and by the superparasitism. Furthermore, the tendency towards decreased parasitism (Figure 15b) as a function of increasing number of hosts available might indicate a depletion of the parasitoid's egg supply at high host:parasitoid ratios and a possible maximum reproductive capacity by the female parasitoid. The greater percentage of parasitized hosts at host:parasitoid ratio of 100:1 (68%) than at the host:parasitoid ratio of 75:1 (60%), does not invalidate the conclusion because both values fall within the 95% confident interval for the regression line (Figure 15b).

Discussion

Increased parasitism with increased host density has been observed in other host:parasitoid systems (Burnet 1958, Podoler and Mendel 1979). Lenteren and Bakker (1978) analyzed the functional response of P. bochei using 3 experimental approaches. They suggested that the decrease in total percentage of parasitism with increasing host density, a tendency that corresponds to the 2nd type of functional response established by Holling (1959b), resulted from an experimental design that forces the

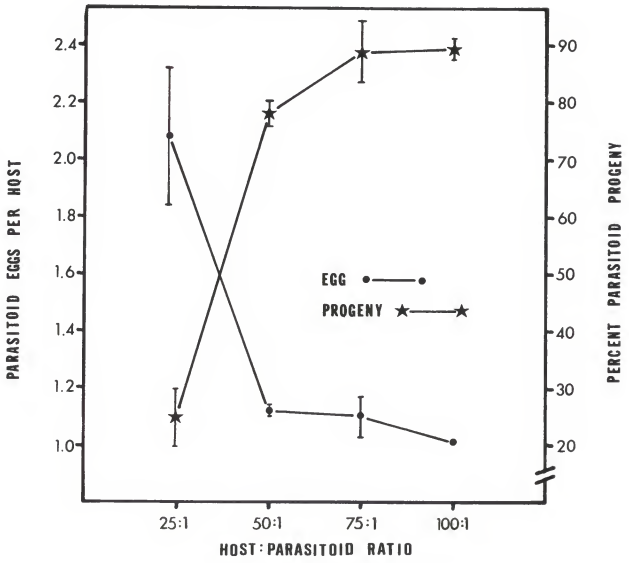
female to remain with hosts in circumstances under which she would normally migrate. An experimental procedure that would restrict the female to a defined space and time, was considered by Lenteren and Bakker (1978) to force her to repeatedly visit areas with low host density or occupied by already parasitized host. These factors distort the actual functional response. The functional response of P. bochei tended to be sigmoidal (Holling 1959b) when the experimental design simulated field situations (Lenteren and Bakker 1978). The same hypothesis may be true for T. daci but different experimental procedures than those used here will be necessary to define the functional response.

To select a proper host:parasitoid ratio for T. daci mass rearing data on the proportion of progeny recovered is valuable. The average yield per female parasitoid at each host density

(Yield = $\frac{\text{Progeny recovered/female/day}}{\text{Egg laid/female/day}}$ X 100) is compared to the number of parasitoid eggs per host in Figure 16. Progeny recovered per day was inversely related to the mean number of eggs per host. The average was significantly lower ($P<0.05$) at the lowest density, in a comparison of the means. The highest averages were found at host densities beyond 200 but they were not significantly different.

An inverse relationship between the mean number of parasitoid eggs and percentage of survival was observed in B. longicaudatus (Lawrence et al. 1978) as a result of superparasitism when the availability of hosts was reduced. In this case the competition between supernumeraries caused reduction in yield. Similar results were reported in Apanteles subandinus Blanch, a parasitoid of the potato tuberworm, Phthorimaea

Figure 16. Distribution of T. daci eggs and percentage of parasitoid yield in 2nd instar A. suspensa larvae at 4 host:parasitoid ratios.


PARASITOID EGGS PER HOST
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
PERCENT PARASITOID PROGENY
90
80
70
60
50
40
30
20
EGG
PROGENY
25:1
50:1
75:1
100:1
HOST:PARASITOID RATIO

operculella (Zeller), (Cardona and Oatman 1975). The reduction of total T. daci progeny at the lowest density resulted from the encapsulation of supernumerary parasitoids. This reaction might be intensified by the stress caused by ovipositing females deprived of hosts. Significantly higher superparasitism ($P<0.05$) was observed at this density (Table 22), and this factor intensified host defenses.

A percentage of hosts, 6.6%, 24.6%, 39.5% and 32% in increasing order of host density, respectively, was left unparasitized at each host density. The mean at the lowest density was significantly lower ($P<0.05$), but no statistical differences were found between the other means at the same level of significance. These data constitute an additional tool in the selection of an economic host:parasitoid ratio for mass rearing. Based on these results it would be advisable to use 50 hosts per female parasitoid for 24 hr exposure. This ratio, however, does not consider the variability in oviposition due to age of females.

The host density had an effect on host mortality. The total host mortality (larvae + pupae) was equal to 35.5, 16.6, 13.8 and 18.8% in increasing order of host density. Normal mortality in non-parasitized hosts was equal to 9.5%. Host mortality indicated that a host:parasitoid ratio lower than 50:1 would represent an economic loss in the mass rearing of T. daci. Results from this experiment agree with previous conclusions. Second instar A. suspensa is a good age for T. daci mass rearing. Based on the parasitoid progeny survival it may be more economical to use a host:parasitoid ratio of 75:1.

SUMMARY AND CONCLUSIONS

Trybliographa daci, a solitary endoparasitoid was reared in 3-day-old A. suspensa larvae. The duration of the cycle of T. daci from egg to adult emergence was 26 - 27 days for males and 28 - 29 days for females at 27.5°C and 50-60% RH. Emergence was asynchronous. The duration and characteristics of T. daci eggs, 4 larval instars, prepupal and pupal stages were described.

The host often encapsulated the parasitoid eggs. Host hemocytes were distributed in an irregular pattern on the parasitoid egg and became melanized within 72 hr after parasitization. The 1st instar parasitoid larvae often remained alive and avoided encapsulation by physical movements and/or repulsion of the host hemocytes. The proportion of T. daci eggs encapsulated was related to the host larval age at the time of parasitization and to the number of parasitoid eggs laid per host. Third instar hosts encapsulated more eggs than 2nd and 1st instar hosts. Superparasitized hosts, of any age, encapsulated more parasitoids than those containing only one parasitoid egg.

Arrhenotoky was the mode of reproduction of T. daci. Virgin females produced only males while mated females gave rise to both males and females. Females mated only once, but they were courted several times. Males mated several times. Females older than 2 days were not courted. The characteristic courtship behavior suggested the involvement of a pheromone.

Newly emerged females had 29 to 32 ovarioles per ovary with 4 or 5 developed eggs per ovariole. This characteristic enabled the female to lay eggs at an early age. In this respect, the high reproductive potential as indicated by the number of ovarioles and number of oocytes is characteristic of ichneumonid parasitoids attacking younger as opposed to older hosts. Thus, it was concluded that T. daci is a parasitoid of younger host larvae rather than older host larvae. When given a choice, female parasitoids preferred to parasitize older hosts. This preference might indicate the importance of host size as a cue for host finding. The proportion of parasitoid progeny produced was related to the age of the host at the time of exposure and significantly larger numbers of parasitoids emerged from younger hosts. Progeny of T. daci varied in size according to the host age, but other morphological characteristics were not affected. The parasitoid developmental time and life span of adults were not affected by the age of the host.

Female T. daci discriminated between parasitized and unparasitized hosts during 24 hr of oviposition. When they were provided with a small number of hosts (viz 12.5 or 25 per female) superparasitism increased. The total number of eggs laid by female parasitoids was inversely proportional to the number of parasitoids present per rearing cage. At high parasitoid:host ratios reduced number of eggs laid resulted from the capacity of parasitoids to restrain oviposition, as well as from interference from other females. The number of parasitoid eggs per host increased with increasing parasitoid density, but the yield of parasitoid progeny decreased as a result of competition between supernumeraries

and/or effect of host defenses. The rate of oviposition per female, number of eggs per host and yield of parasitoid progeny at different parasitoid densities followed the same tendency in 2nd and 3rd instar hosts. The yield, however, of the parasitoid progeny increased with increasing 2nd instar host density.

Second instar host larvae were physiologically suitable and economically acceptable for mass rearing of T. daci. The 2nd instar hosts could be parasitized in the host diet, thus reducing labor costs. The best host:parasitoid ratio for 24 hr of exposure was 75:1. Hosts can be presented to parasitoids at 2 or 3 days of age in order to obtain maximum number of parasitoid production for colony maintenance. T. daci developed within the same range of temperature and RH, used to rear A. suspensa, and a temperature of 26 - 27°C, 70-80% RH and 50% water (W/W) in the pupation medium should be maintained during the whole cycle.

Additional research is needed in several areas as a result of these studies. Major areas requiring work are related to the process of encapsulation and evasion of host defenses, reproductive potential and life table studies. Specific areas for investigation might include the following:

a. Histological and physiological studies of the capsule formation and changes in the host hemocytes following parasitization.

b. Relationship between age and experience of the female parasitoid and incidence of encapsulation.

c. Effect of external factors, such as temperature and RH and intrinsec factors, such as host strain, host diet, on the incidence of encapsulation.

d. Mechanisms of intraspecifc competition between supernumerary parasitoids.

REFERENCES

Ahmad, R., M. Murtaza, S. Caleb, and R.A. Syed. 1972. Note on breeding parasites of fruit flies in Pakistan. FAO Plant Protection Bull. 146-147.

Alphen, J.J.M. van. 1980. Aspects of foraging behavior of Tetrasticus asparagi Crawford and Tetrasticus sp. (Eulophidae), gregarious parasitoids of the asparagus beetles Crioceris asparagi L (Chrysomelidae). Neth. J. Zool. 30: 307-325.

Ashley, T.R., and L. Chambers. 1979. Effects of parasite density and host availability on progeny production by Biosteres (Opius) longicaudatus (Hymenoptera: Bracondidae), a parasite of Anastrepha suspensa (Diptera: Tephritidae). Entomophaga 24: 363-369.

Ashley, T.R., P.D. Greany, and L. Chambers. 1976. Adult emergence in Biosteres (Opius) longicaudatus and Anastrepha suspensa, in relation to the temperature and moisture concentration of the pupation medium. Fla. Entomol. 59: 391-396.

Ashmead, W.H. 1903. Classification of the gall-wasps and the parasitic cynipoids of the superfamily Cynipoidea. Psyche 10: 59-73.

Askew, R.R. 1971. Parasitic Insects. American Elsevier Pub. Co., Inc. New York. 316 pp.

Assem, J. van den. 1969. Reproductive behavior of Pseudeucoila bochei (Hymenoptera: Cynipidae). I. A description of courtship behavior. Neth. J. Zool. 19: 641-648.

Bakker, K. 1971. Host selection on Pseudeucoila bochei. Proc. XIII Int. Cong. Entomol. Moscow 1: 472.

Bakker, K., S.N. Bagchee, W.R. Van Zwet, and E. Meelis. 1967. Host discrimination in Pseudeucoila bochei (Hymenoptera: Cynipidae). Entomol. Exp. Appl. 10: 295-311.

Baranowski, R.M., and R.W. Swanson. 1970. Introduction of Parachasma (= Opius) cereus (Hymenoptera: Braconidae) into Florida as a parasite of Anastrepha suspensa (Diptera: Tephritidae). Fla. Entomol. 53: 61-62.

Baranowski, R.M. and R.W. Swanson. 1971. The utilization of Parachasma cereum (Hymenoptera: Braconidae) as a means of suppressing Anastrepha suspensa (Diptera: Tephritidae) populations. Proc. Tall Timbers Conf. on Ecological Animal Control by Habitat Management. 3: 249-252.

Beckage, N.E., and L.M. Riddiford. 1978. Developmental interactions between the tobacco hornworm Manduca sexta and its braconid parasite Apanteles congregatus. Entomol. Exp. Et. Appl. 23: 139-151.

Beland, G.L., and E.G. King. 1976. South western corn borer: suitability of larval stages for development of the tachinid parasite, Lixophaga diatraeae. Environ. Entomol. 5: 421-426.

Bess, H.A., R. van den Bosch, and F.H. Haramoto. 1961. Fruit fly parasites and their activity in Hawaii. Hawaii. Entomol. Soc. Proc. 17: 367-378.

Boush, G.M., and R.J. Baerwald. 1976. Courtship behavior and evidence for a sex pheromone in the apple maggot parasite, Opius alloeus (Hymenoptera: Braconidae). Ann. Entomol. Soc. Amer. 60: 865-868.

Brown, W.L., Jr., T. Eisner, and R.H. Whittaker. 1970. Allomones and kairomones: transpecific chemical messengers. Bioscience 20: 21-2.

Burditt, A.K., Jr., F. Lopez, L.F. Steiner, D.L. von Windeguth, R.M. Baranowski, and M. Anwar. 1974. Application of Sterilization Techniques to Anastrepha suspensa Loew in Florida, United States of America. In Sterile Principle for Insect Control. Symp. Proc. Innsbruck, 22-26 July, 1974. Int. Atomic Energy Agency. Vienna, 1975.

Burnet, T. 1958. Effects of host distribution on the reproduction of Encarsia formosa Gaham (Hymenoptera: Chalcidoidea). Can. Entomol. 90: 179-191.

Bursell, E. 1964. The Insect and the External Environment. I. In Physiology of Insecta. M. Rockstein, ed. Academic Press. New York/London. 1, 640 pp.

Cardona, C., and E.R. Oatman. 1975. Biology and physical ecology of Apanteles subandinus Blanchard (Hymenoptera: Braconidae), with notes on temperature responses of Apanteles scutellaris Muesebeck and its host, the potato tuberworm. Hilgardia 43: 1-51.

Chabora, P.C., and D. Pimentel. 1966. Effect of host (Musca domestica L.) age on the pteromalid parasite Nasonia vitripennis Walker. Canad. Entomol. 98: 1226-1231.

Chabora, P.C., S.J. Smolin, and A.H. Kopelman. 1979. The life history of Pseudeucoila sp. a protelian parasite of Drosophila. Ann. Entomol. Soc. Amer. 72: 495-499.

Chambers, D.L. 1977. Situacion actual de la mosca de la fruta del caribe Anastrepha suspensa Loew. Folia Entomol. Mexicana 34: 25-35.

Chapman, R.F. 1971. The insects, structure and function. Elsevier. N York. 819 pp.

Christenson, L.D., and R.H. Foote. 1960. Biology of fruit flies. Ann. Rev. Entomol. 5: 171-192.

Clancy, D.W. 1950. Notes on parasites of tephritid flies. Hawaii. Entomol. Soc. 14: 23-28.

Clancy, D.W. 1952. Notes on parasites of fruit flies. Hawaii. Entomol. Soc. Proc. 14: 371-375.

Clancy, D.W., P.E. Marucci, and E. Dresner. 1952. Importation of natural enemies to control the oriental fruit fly in Hawaii. J. Econ. Entomol. 45: 85-90.

Clark, A.M., and M. Rockstein. 1964. Aging in Insects. In The Physiology of Insecta. M. Rockstein, ed. Academic Press. New York/ London. 1, 640.

Clausen, C.P. 1939. The effect of host size upon the sex ratio of Hymenopterous parasites and its relation to methods of rearing and colonization. N. Y. Entomol. Soc. 47: 1-19.

Clausen, C.P. 1940. Entomophagous Insects. McGraw-Hill Book Co., Inc. New York. 688 pp.

Clausen, C.P., D.W. Clancy,, and Q.C. Chock. 1965. Biological control of the oriental fruit fly (Dacus dorsalis Hendel) and other fruit flies in Hawaii. U.S.D.A. Tech. Bull. 1322. 102 pp.

Coats, S.A. 1976. Life cycle and behavior of Miscidifurax zaraptor (Hymenoptera: Pteromalidae). Ann. Entomol. Soc. Amer. 69: 772-780.

Corbet, S.A., and S. Rotheran. 1965. The life history of the Ichneumonid Nemeristis canescens (Gravenhorst) as a parasite of the Mediterranean flour moth Ephestia kueniella Zeller under laboratory conditions. Proc. Roy. Entomol. Soc. London. (A). 40: 67-72.

Deevey, S.E. 1947. Life tables for natural populations of animals. Quart. Rev. Biol. 22: 283-314.

Dodson, G. 1978. Morphology of the reporductive system in Anastrepha suspensa and notes on related species. Fla. Entomol. 61: 231-239.

Doutt, R.L. 1964. Biological Characteristics of Entomophagous Adults. In Biological Control of Insect Pest and Weeds. P. DeBock, ed. Reinhold Pub. Corp. New York. 844 pp.

Doutt, R.L., D.P. Annecke, and E. Tremblay. 1976. Biology and Host Relationships of Parasitoids. In Theory and Practice of Biological Control. C.B. Huffaker and P.S. Messenger, eds. Academic Press. New York. 788 pp.

Edson, K.M., S.B. Vinson, D.B. Stoltz, and M.D. Summers. 1980, Virus in a parasitoid wasp: suppression of the cellular immune response in the parasitoid host. Science 211: 582-583.

Eijsacker, H.J.P., and K. Bakker. 1971a. Duration of the egg stage of the sexes in Pseudeucoila bochei Welder (Cynipidae) a parasite of Drosophila larvae. Neth. J. Zool. 21: 166-171.

Eijsacker, H.J.P., and K. Bakker. 1971b. Elimination by physical attack of supernumerary larvae of Pseudeucoila bochei Weld (Cynipidae) in their hosts, larvae of Drosophila. Neth. J. Zool. 21: 205-107.

Eijsacker, H.J.P., and J.C. van Lenteren. 1970. Host choice and host discrimination in Pseudeucoila bochei (Hymenoptera: Cynipidae) Neth. J. Zool. 20: 414.

Fisher, R.C. 1961. A study in multiparasitism. II. The mechanism and control for possession of the host. J. Exp. Biol. 38: 605-628.

Fisher, R.C. 1965. The physiological suppression of insect parasitoids. Poc. Inter. Cong. Ent. London. 12: 413.

Fisher, R.C. 1971. Aspects of the physiology of endoparasitic hymenoptera. Biol. Rev. 46: 243-278.

Fisher, R.C., and V.K. Ganesalingam. 1970. Changes in the composition of host haemolymph after attack by an insect parasitoid. Nature 227: 191-192.

Fiske, W.F. 1910. Superparasitism; an important facter in natural control of insects. J. Econ. Entomol. 3: 88-97.

Flanders, S.E. 1935. Effects of host density on parasitism. J. Econ. Entomol. 28: 889-900.

Flanders, S.E. 1942. Oosorption and ovulation in relation to oviposition in the parasitic Hymenoptera. Ann. Entomol. Soc. Amer. 35: 251-266.

Greany, P.D., H.R. Agee, A.K. Burditt, and D.L. Chambers. 1977a. Field studies on color preferences of the Caribbean fruit fly, Anastrepha suspensa (Diptera: Tephritidae). Entomol. Exp. Appl. 21: 63-70.

Greany, P.D., T.R. Ashley, R.M. Baranowski, and D.L. Chambers. 1976. Rearing and life history studies on Biosteres (Opius) longicaudatus (Hymenoptera: Braconidae). Entomophaga 21: 207-205.

Greany, P.D., A.K. Burditt, Jr., H.R. Agee, and D.L. Chambers. 1978. Increasing effectiveness of visual traps for the Caribbean fruit fly, Anastrepha suspensa (Diptera: Tephritidae). Entomol. Exp. Appl. 23: 20-25.

Greany, P.D., S.D. Hawke, T.C. Carlysle, and D.W. Anthony. 1977b. Sense organs in the ovipositor of Biosteres (Opius) longicaudatus, a parasite of the Caribbean fruit fly Anastrepha suspensa. Ann. Entomol. Soc. Amer. 70: 319-321.

Greany, P.D., J.H. Tumlinson, D.L. Chambers, and G.M. Boush. 1977c. Chemically mediated finding by Biosteres longicaudatus, a parasitoid of tefritid fruit fly larvae. J. Chem. Ecol. 3: 189-195.

Greene, C.T. 1934. A revision of the genus Anastrepha based on the wings and on the length of the ovipositor sheath (Diptera: Tephritidae). Proc. Entomol. Soc. Wash. 36: 127-179.

Gutierrez, A.P. 1970. Studies on host selection and host specificity of the hyperparasite Charips victris. III. Host suitability studies. Ann. Entomol. Soc. Amer. 63: 1485-1491.

Hagen, H.S. 1964. Developmental Stages of Parasites. In Biological Control of Insect Pest and Weeds. Reinhold Pub. Corp. New York. 844 pp.

Hamilton, W.D. 1967. Extraordinary sex ratios. Science 156: 476-487.

Havilan, M.D. 1921. On the bionomics and post-embryonic development of certain cynipids hyperparasites of aphids. Quart. J. Microscop. Sci. 65: 451-478.

Holling, C.S. 1959a. The components of predation as revealed by a study of small mammals predation of the european pine sawfly. Can. Entomol. 91: 293-320.

Holling, C.S. 1959b. Some characteristics of simple types of predation and parasitism. Can. Entomol. 91: 385-398.

Imms, A.D. 1951. A general Textbook of Entomology. Chapman and Hall Ltd. London. 9th edition. 886 pp.

James, H.C. 1928. On the life histories and economic status of certain cynipids parasites of dipterous larvae, with description of some new larval forms. Ann. Appl. Biol. 15: 287-316.

Jenni, W. 1951. Beitrage zur Morphologie und Biologie der Cynipidae Pseudeucoila bochei Weld. eines larven parasiten von Drosophila melanogaster. Meig. Acta Zool. Stockholm. 32: 117-254.

Keilin, D. et de la Baume-Pluvinel. 1913. Formes larvaries et biologie d'un cynipide entomophage Eucoila kelini kieffer. Bull. Scientif de France et Belgique. Bd. 47:

Kitano, H. 1969. Experimental studies on the parasitism of Apanteles glomeratus L. with special reference to encapsulation-inhibiting capacity. Bull. Tokyo Gekugei Univ. 21: 95-136.

Kopelman, A.H., and P. C. Chabora. 1978. Resource availability and reproductive variation in Pseudeucoila sp. (Hymenoptera: Cynipidae). N.Y. Entomol. Soc. 86: 302.

Korytkowsky, Ch., y D. Ojeda. 1968. Especies del genero Anastrepha Schiner en el noreste peruano. Revista Peruana de Entomologia 2: 32-70.

Lawrence, P.O. 1979. Immature stages of the Caribbean fruit fly Anastrepha suspensa. Fla. Entomol. 62: 214-219.

Lawrence, P.O. 1981a. Host vibration--a cue to host location by the parasite, Biosteres longicaudatus. Oecologia. Berlin. 48: 249-251.

Lawrence, P.O. 1981b. Interference competition and optimal host selection in the parasitic wasp, Biosteres longicaudatus. Ann. Entomol. Soc. Amer. 74: 540-544.

Lawrence, P.O., R.M. Baranowski, and P.D. Greany. 1976. Effects of host age on development of Biosteres (=Opius) longicaudatus, a parasitoid of the Caribbean fruit fly, Anastrepha suspensa. Fla. Entomol. 59: 33-39.

Lawrence, P.O., P.D. Greany, J.L. Nation, and R.M. Baranowski. 1978. Oviposition behavior of Biosteres longicaudatus a parasite of the Caribbean fruit fly Anastrepha suspensa. Ann. Entomol. Soc. Amer. 71: 253-256.

Legner, E.F. 1969. Adult emergence interval and reproduction in parasitic Hymenoptera influenced by host size and density. Ann. Entomol. Soc. Amer. 62: 220-226.

Lenteren, J.C. van. 1972. Contact-chemoreceptors on the ovipositor of Pseudeucoila bochei Weld. (Cynipidae). Neth. J. Zool. 22: 347-350.

Lenteren, J.C. van 1976a. The development of host discrimination and prevention of superparsitism in the parasite Pseudeucoila bochei Weld. (Hymenoptera: Cynipidae). Nether. J. Zool. 26: 1-83.

Lenteren, J.C. van. 1976b. Discrimination between parasitized and unparasitized hosts in the parasitic wasp Pseudeucoila bochei: a matter of learning. Nature 253: 417-419.

Lenteren, J.C. van, and J.M. van Alphen. 1978. How to analyze host discrimination. Ecol. Entomol. 3: 71-75.

Lenteren, J.C. van, and K. Bakker. 1978. Behavioral aspects of functional response of a parasite (Pseudeucoila bochei Weld.) to its host (Drosophila melanogaster). Neth. J. Zool. 28: 213.233.

Lewis, W.J., and S. Vinson. 1971. Suitability of certain Heliothis (Lepidoptera: Noctuidae) as host for the parasite Cardiochiles nigriceps. Ann. Entomol. Soc. Amer. 64: 970-972.

Lynn, D.C., and S.B. Vinson. 1967. Effects of temperature, host age, and hormones upon encapsulation of Cardiochiles nigriceps eggs by Heliothis spp. J. Invertebr. Pathol. 29:50-55.

Machin, J. 1979. Atmospheric Water in Arthropods. In Advances in Insect Physiology. J.E. Threherne, M.J. Berridge and V.B. Wigglesworth, eds. Academic Press. London/New York. 437 pp.

Marucci, P.E., and D.W. Clancy. 1950. The artificial culture of fruit flies and their parasites. Hawaii. Entomol. Soc. Proc. 14: 163-166.

Mazomenos, B., J.L. Nation, W.J. Coleman, K.C. Dennis, and R. Esponda. 1977. Reproduction in the Caribbean fruit fly: comparison between a laboratory and a wild strain. Fla. Entomol. 60: 139-144.

Miles, L.R., and E.G. King. 1975. Development of the tachinid parasite, Lixophaga diatraeae on various developmental stages of the sugarcane borer in laboratory. Environ. Entomol. 4: 811-814.

Morgante, J.S., A. Malvasi, and G.L. Bush. 1980. Biochemical systematics and evolutionary relationship of neotropical Anastrepha. Ann. Entomol. Soc. Amer. 73: 622-630.

Nappi, A.J. 1973. Cellular immune reaction of Drosophila algonquin against Pseudeucoila bochei. Amer. Zool. 23: 1295.

Nappi, A.J. 1975a. Parasite Encapsulation in Insects. In Invertebrate Immunity. K. Maramorosch and R.E. Shope, eds. Academic Press. New York. 365 pp.

Nappi, A.J. 1975b. Cellular immune reactions of larvae of Drosophila algonquin. Parasitol. 70: 189-194.

Nappi, A.J., and J.G. Stofolano. 1971. Heterotylenchus automalis, haemocytic reactions and capsule formation in the host Musca domestica. Exp. Parasitol. 29: 116-125.

Nappi, A.J., and F.A. Streams. 1969. Haemocytic reaction of Drosophila melanogaster to the parasites Pseudeucoila mellipes and P. Bochei. J. Insect. Physiol. 15: 1551-1566.

Nation, J.L. 1972. Courtship behavior and evidence for a sex attractant in the male Caribbean fruit fly, Anastrepha suspensa. Ann. Entomol. Soc. Amer. 65: 1364-1367.

Nation, J.L. 1975. The sex pheromone blend of Caribbean fruit fly males: isolation, biological activity and partial chemical characterization. Environ. Entomol. 4: 27-30.

Nation, J.L. 1982. Sex pheromone of the Caribbean fruit fly; chemistry and field ecology. Proc. Fifth Inter. Cong. of Pesticide Chem. Japan. (In press.)

Nordlund, D.A., and W.J. Lewis. 1976. Terminology of chemical releasing stimuli in intraspecific interactions. J. Chem. Ecol. 2: 211-220.

Pemberton, C.E., and H.F. Willard. 1918. A contribution to the biology of fruit fly parasites in Hawaii. J. Agricul. Res. 15: 419-466.

Perdomo, A.J., R.M. Baranowski, and J.L. Nation. 1975. Recapture of virgin female Caribbean fruit flies from traps baited with males. Fla. Entomol. 58: 291-296.

Perdomo, A.J., J.L. Nation, and R.M. Baranowski. 1976. Attraction of female and male Caribbean fruit flies to food-baited and male-baited traps under field conditions. Environ. Entomol. 5: 1208-1210.

Podoler, H., and Z. Mendel. 1979. Analysis of a host-parasite (Ceratitis-Muscidifurax) relationship under laboratory conditions. Ecol. Entomol. 4: 45-59.

Prescot, J.A., and R.M. Baranowski. 1971. Effects of temperature on the immature stages of Anastrepha suspensa (Diptera: Tephritidae). Fla. Entomol. 54: 297-303.

Price, P.W. 1972. Parasitoids utilizing the same host: adaptative nature of differences in size and form. Ecology 53: 190-195.

Price, P.W. 1973. Reproductive strategies in parasitoid wasps. Amer. Natur. 107: 684-693.

Puttler, B. 1961. Biology of Hyposoter exiguae (Hymenoptera: Ichneumonidae), a parasite of lepidopterous larvae. Ann. Entomol. Soc. Amer. 54: 25-30.

Puttler, B., and R. van den Bosch. 1959. Partial immunity of Laphygma exigua (Hubner) to the parasite Hyposoter exiguae (Viedereck). J. Econ. Entomol. 52: 327-329.

Ratcliffe, N.A., and A.F. Rowley. 1979. Role of Hemocytes in Defense Against Biological Agents. In Insect Hemocytes. A.P. Gupta, ed. Cambridge Univ. Press. London/New York. 614 pp.

Riddiford, L.M. 1975. Host Hormones and Insect Parasites. In Invertebrate Immunity. K. Maramorosch and R.E. Shope, eds. Academic Press. New York. 365 pp.

Rizki, M.T.M. 1957. Alterations in the haemocyte population of Drosophila melanogaster. J. Morph. 100: 437-458.

Rosen, D., and P. DeBach. 1973. Systematics, morphology and biological control. Entomophaga 18: 215-222.

Rotheram, S. 1967. Immune surface of eggs of a parasitic insect. Nature. London. 214: 700.

Rotheram, S. 1973. The surface of the egg of a parasitic insect. II. The ultra structure of the particulate coat on the egg of Nemeristis. Proc. Roy. Soc. London. (B). 183: 195-204.

Rotheray, G.E. 1979. The biology and host searching behavior of a cynipid parasite of aphidophagous syrphid larvae. Ecol. Entomol. 4: 75-82.

Rotheray, G.E. 1981. Host searching and oviposition behavior of some parasitoids of aphidophagous syrphidae. Ecol. Entomol. 6: 79-87.

Salt, G. 1934. Experimental studies in insect parasitism. II. Superparasitism. Proc. Roy. Soc. London. (B). 114: 455-476.

Salt, G. 1935. Experimental studies in insect parasitism. III. Host selection. Proc. Roy. Soc. London. (B). 117:413-435.

Salt, R. 1937. The sense used by Trichogramma to distinguish between parasitized and unparasitized hosts. Proc. Roy. Soc. London. (B). 122: 57-75.

Salt, G. 1938. Experimental studies in insect parasitism. VI. Host suitability. Bull. Entomol. Res. 29: 223-246.

Salt, G. 1941. The effects of host upon their insect parasites. Biol. Rev. 16: 239-264.

Salt, G. 1961. Competition among insect parasitoids. Symp. Doc. Exp. Biol. 15: 96-119.

Salt, G. 1963. The defense reactions of insects to metazoan parasites. Parasitol. 53: 527-642.

Salt, G. 1968. The resistance of insect parasitoids to the defense reaction of their hosts. Biol Rev. 43: 200-232.

Salt, G. 1970. The cellular defense reaction of insects. Cambridge Univ. Press. London/New York. 118 pp.

Salt, G. 1973. Experimental studies in insect parasitism. XVI. The mechanism of the resistance of Nemeristis to defense reactions. Proc. Roy. Soc. London. (B). 183: 337-350.

Schilinger, E.I., and J. Hall. 1961. The biology, behavior and morphology of Trioxys (Trioxys) utilis, an internal parasite of the spotted alfalfa aphid, Therioaphis maculata (Hymenoptera: Braconidae, Aphidiinae). Ann. Entomol. Soc. Amer. 54: 34-45.

Short, J.R.T. 1952. The morphology of the head of larval Hymenoptra with special reference to the head of Ichneumonoidea, including a classification of the final instar larvae of the Braconidae. Roy Entomol. Soc. London. Trans. 103: 27-84.

Simmonds, F.J. 1952. Parasites of the fruit fly Oscinella frit (L.) in eastern North America. Bull. Entomol. Res. 43: 503-542.

Southwood, T.R.E. 1978. Ecological methods, with particular reference to the study of insect populations. 2nd edition. The Univ. Printing House. Cambridge. 542 pp.

Steyskal, G.C. 1977. Pictorial key to species of the genus Anastrepha (Diptera: Tephritidae). Entomol. Soc. Washington. 35 pp.

Stoltz, D.B., and B.S. Vinson. 1976. Baculovirus-like particles of female parasitoid wasps II: the genus Apanteles. Can. J. Microbiol. 23: 28-37.

Stoltz, D.B., S.B. Vinson, and E.A. MacKinnon. 1976. Baculovirus-like particles in the reproductive tracts of female parasitoid wasps. Canad. J. Microbiol. 22: 1013-1023.

Stone, A. 1942. The fruit flies of the genus Anastrepha. U.S.D.A. misc. pub. 439. 112 pp.

Streams, A.F. 1968. Factors affecting the susceptibility of Pseudeucoila bochei eggs to encapsulation by Drosophila melanogaster. J. Invertebr. Pathol. 12: 379-387.

Streams, A.F. 1971. Encapsulation of insect parasites in superparasitized hosts. Entomol. Exp. et Appl. 14: 484-490.

Streams, A.F., and L. Greenberg. 1969. Inhibition of the defense reactions of Drosophila melanogaster parasitized simultaneously by the wasps Pseudeucoila bochei and Pseudeucoila mellipes. J. Invertebr. Pathol. 13: 371-377.

Swanson, R.W., and R.M. Baranowski. 1972. Host range and infestation by the Caribbean fruit fly Anastrepha suspensa (Diptera: Tephritidae) in Florida. Fla. State Hort. Soc. Proc. 85: 271-274.

Takei, G.H., and M. Tamashiro. 1980. The apparent methamorphosis and related defense reactions of haemocytes on the lawn armyworm, Spodoptera mauritia acrocynctoides (Guenee). Hawaii. Agric. Exp. Sta. Univ. of Hawaii Tech. Bull. 104. 40 pp.

Veerkamp, F.A. 1980. Behavioral differences between two strains and hybrids of the wasp Pseudeucoila bochei Weld (Hymenoptera: Cynipidae), a parasite of Drosophila melanogaster. Neth. J. Zool. 30: 431-449.

Vinson, S.B. 1972a. Courtship behavior and evidence for sex pheromone in the parasitoid Campoletis sonorensis (Hymenoptera: Ichneumonidae). Environ. Entomol. 1: 409-414.

Vinson, S.B. 1972b. Factor involved in successful attack on Heliothis virescens by the parasitoid Cardichiles nigriceps. J. Invertebr. Pathol. 20: 118-123.

Vinson, S.B. 1975. Biochemical Coevolution Between Parasitoids and Their Hosts. In Evolutionary Strategies of Parasitic Insects and Mites. P. Price, ed. Plenum Press. New York. 225 pp.

Vinson, S.B. 1976. Host selection in insect parasitoids. Ann. Rev. Entomol. 21: 109-133.

Vinson, S.B. 1977a. Behavioral Chemical in The Augmentation of Natural Enemies. In Biological Control by Augmentation of Natural Enemies; Insect and Mite Control with Parasite and Predators. R.L. Ridway and S.B. Vinson, eds. Plenum Press. New York. 480 pp.

Vinson, S.B. 1977b. Insect Host Responses Against Parasitoid Resistance: With Emphasis on Lepidoptera-Hymenoptera Association. In Comparative Phathobiology. L.A. Bulla and T.C. Cheng, eds. Plenum Pub. Corp. New York. 3, 192 pp.

Vinson, S.B. 1980. Host suitability for insect parasitoids. Ann. Rev. Entomol. 25: 293-419.

Vinson, S.B., and D.J. Barras. 1970. Effects of the parasitoid, Cardiochiles nigriceps on the growth, development and tissues of Heliothis virescens. J. Insect Physiol. 16: 1329-1338.

Vinson, S.B., and G.F. Iwantsch. 1980. Host regulation by insect parasitoids. Quart. Rev. Biol. 55: 143-165.

Vinson, S.B., and J.R. Scott. 1974. Parasitoid egg shell changes in suitable and unsuitable hosts. J. Ultrastructure Res. 47: 1-15.

Vinson, S.B., and J.R. Scott. 1975. Particles containing DNA associated with the oocyte of an insect parasitoid. J. Invertebr. Pathol. 25: 375-378.

Wadley, F.M. 1967. Experimental statistics in entomology. Grad. School Press. U.S.D.A. Washington, D.C. 133 pp.

Walker, I. 1959. Die abwehrreaktion des wirtes Drosophila melanogaster gegen die zoophage cynipidae Pseudeucoila bochei Weld. Rev. Suisse. Zool. 66: 569-632.

Weber, P.W. 1951. Recent liberations of beneficial insects in Hawaii. Hawaii. Entomol Soc. Proc. 14: 327-330.

Weems, H.J., Jr. 1965. Anastrepha suspensa (Loew) (Diptera: Tephritidae). Fla. Dept. of Agri. Div. of Plant Industry. Entomol. Cir. 38. 4 pp.

Weld, L.H. 1951. A new species of Trybliographa (Hymenoptera: Cynipidae). Hawaii. Entomol. Soc. Proc. 14: 331-332.

Whitcomb, R.F., Shapiro, M., and R.R. Granados. 1974. Insect Defense Mechanisms Against Microorganisms and Parasitoids. In The Physiology of Insecta. M. Rockstein, ed. Vol. 5. Academic Press. New York. 648 pp.

Wishart, G., and E. Monteith. 1954. Trybliographa rapae (Westw.). (Hymenoptera: Cynipidae), a parasite of Hylemyia spp. (Diptera: Anthomyiidae). Canad. Entomol. 8: 145-154.

Yoshida, S. 1978. Behavior of males in relation to the female sex pheromone in the parasitoid wasp Anisopteromalus calandrae (Hymenoptera: Pteromalidae). Entomol. Exp. et Appl. 23: 152-162.

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Ligia Nunez-Bueno was born on April 2, 1940, in San Andres, Santander, Colombia. She attended primary and secondary schools in Colombia, and received the degree of Licenciada from Universidad Pedagogica de Colombia in Bogota in 1964. She enrolled in the Instituto Tecnologico y de Estudios Superiores de Monterrey, in Mexico in January 1971 and received the Master of Science degree in April 1973. The economical support during this time came from a personal loan from the government of Colombia through ICETEX. She worked as an entomologist at the Division of Sanidad Vegetal at Instituto Colombiano agropecuario from September 1973 to March 1979. She was in charge of a national fruit fly survey program.

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She enthusiastically hopes to continue working in Colombia with the positive idea of contributing to the progress of her country.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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